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(54) Title: SKIN CONDITIONING COMPOSITIONS CONTAINING COMPOUNDS FOR MIMICKING THE EFFECT ON SKIN OF RETINOIC ACID

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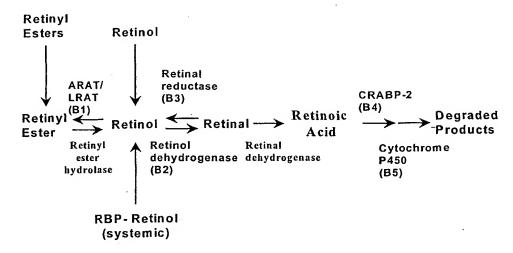
SKIN CONDITIONING COMPOSITIONS CONTAINING COMPOUNDS FOR MIMICKING THE EFFECT ON SKIN OF RETINOIC ACID

The present invention relates to cosmetic skin conditioning compositions containing certain compounds which mimic the effect on skin of retinoic acid.

Retinol (vitamin A) is an endogenous compound which occurs naturally in the human body, and is essential for normal epithelial cell differentiation. Natural and synthetic 10 vitamin A derivatives have been used extensively in the treatment of a variety of skin disorders and have been used as skin repair or renewal agents. Retinoic acid has been employed to treat a variety of skin conditions, e.g., acne, wrinkles, psoriasis, age spots and discoloration. 15 See e.g., Vahlquist, A. et al., J. Invest. Dermatol., Vol. 94, Holland D.B. and Cunliffe, W.J. (1990), pp. 496-498; Ellis, C.N. et al., "Pharmacology of Retinols in Skin", Vasel, Karger, Vol. 3, (1989), pp. 249-252; Lowe, N.J. et al., "Pharmacology of Retinols in Skin", Vol. 3, (1989), pp. 240-248; PCT Patent 20 Application No. WO 93/19743.

It is believed that retinol esters and retinol are enzymatically converted in the skin into retinoic acid according to the following mechanism:

Retinol metabolism in the epidermis: enzyme names



The present invention is based on the discovery that certain compounds enhance the conversion of retinyl esters and retinol to retinoic acid. The compounds are collectively termed "boosters" and are coded as groups B1 to B5 according to the boosting mechanism of the particular compound. mechanism of the booster groups is as follows: inhibiting (AcylCoenzymeA(CoA): retinol ARAT/LRAT transferase/Lecithin: retinol acyl transferase) activity 10 activity enhancing retinol dehydrogenase inhibiting retinal reductase activity (B3), antagonising CRABP-II (Cellular Retinoic Acid Binding Protein II) binding retinoic acid (B4) and inhibiting cytochrome dependent retinoic acid oxidation (B5). 15

The boosters alone or in combination with each other potentiate the action of retinoids by increasing the conversion of the retinoids to retinoic acid and preventing

the degradation of retinoic acid. The boosters act in conjunction with a retinoid (e.g. retinol, retinyl esters, retinal, retinoic acid), the latter being present endogenously in the skin. The preferred compositions, however, include a retinoid in the composition, co-present with a booster or a combination of boosters, to optimise performance.

Several patents by Granger et al describe the use retinoid boosters in cosmetic compositions to improve the 10 efficacy of retinol and retinyl esters (US patent numbers: 5747051,5716627, 5811110, 5759556, 5756109, 5747051, 5599548, 5955092, 5885595, 5759556, 5693330). boosters described in these patents are restricted to class Furthermore Johnson & Johnson have a series of 15 patents which describe the use of molecules which fall into class 5 booster molecules (U.S. 5028628; U.S. 5037829; U.S. 5151421; U.S. 476852; U.S. 5500435; U.S. 5583136; U.S. 5612354).

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The molecules which act as retinoid boosters are common ingredients in cosmetic products. There is considerable prior art describing their use in cosmetic compositions. There is substantial prior art describing the use of two or more of these molecules in the same composition. Some of the examples of the prior art are as in US 5,665,367, US 5747049, US 5853705, US 5766575, and US 5849310.

However, the prior art does not describe synergy arising from combinations of booster molecules. This observation of a synergistic boosting of retinoid activity from

combinations of booster molecules was an unexpected finding. The prior art does not describe optimal concentrations or ratios of booster molecules or ratios of booster molecules to that of retinoids. Thus, the present invention is novel in that by combining cosmetic retinoids with booster molecules, at the most appropriate concentrations or ratios, a substantial improvement in efficacy of the retinoids is obtained.

10 The classes of boosters suitable for use in the present invention include but are not limited to the boosters listed in Tables B1 through to B5.

Best Groups of Boosters

B1 Compounds

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1. Fatty Acid Amides	These are readily commercially
-	available and have the added
	advantage of being surfactants and
	thus help generate emulsions
	suitable for cosmetic preparations.
2. Ceramides	These can additionally act as
	precursors of stratum corneum
	barrier ceramides.
3. Carotenoids	These can offer some UV protection
	and act as natural colorants.
4. Flavanoids	Natural antioxidants.
5. Cyclic fragrances	These are readily commercially
	available and additionally can be
	used to fragrance the product.
6. Non-cyclic	These can be used to fragrance the
fragrances	product.
7. Phospholipid	These can be utilised by skin cells
analogues	to nourish the generation of
	barrier components.
8. Ureas	These are readily commercially
	available and can also act as
	preservatives for the product.

B2 Compounds

1. Phosphatidyl choline	Most preferred as most active
	activator of Retinol
	Dehydrogenase
2. Sphingomyelin	

5 B3 Compounds

Arachidonic Acid Linoleic Acid	Fatty Acids which can be useful in maintaining stratum
Linolenic Acid	corneum barrier
Myristic Acid	
Linoleic Acid	Essential Fatty Acids
Linolenic Acid	
Arachidonic Acid	Non-essential fatty acids
Myristic Acid	
Glycyrrhetinic Acid	Polycyclic triterpene
	carboxylic acid which is
	readily obtained from plant
	sources.
Phosphatidyl ethanolamine	Can be incorporated into
·	cellular membranes.

B4 Compounds

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Hexadecanedioic acid	Saturated fatty acids.
12-hydroxystearic acid	
Isostearic acid	
Linseed oil	Unsaturated fatty acids
Elaidic acid	
Elaidic acid	Solid at room temperature
Isostearic acid	
Hexadecanedioic acid	
Linseed oil	Liquid at room temperature
12-hydroxystearic acid	

B5 Compounds

Bifonazole Climbazole Clotrimazole Econazole Ketoconazole Miconazole	Antimicotics
Climbazole Lauryl hydroxyethylimidazoline	Readily commercially available Compounds which are readily commercially available and have the added advantage of being surfactants and thus help generate emulsions suitable for cosmetic preparations.
Quercetin	Naturally occuring flavanoid which has antioxidant properties.
Coumarin	Natural colorant
Quinolines Isoquinolines	·
Metyrapone	

5 The present invention includes, in part, a skin conditioning composition containing from about 0.0001% to about 50%, preferably from 0.001% to 10%, most preferably from 0.001% to 5% by weight of the composition of a booster or combination of boosters and a cosmetically acceptable vehicle.

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The boosters or combinations thereof included in the inventive compositions are selected from the group consisting of:

- (a) a booster selected from the group consisting of B2; B3; B4;
 - (b) binary combinations of boosters selected from the group consisting of:

B1/B2; B1/B3; B1/B4; B1/B5; B2/B3, B2/B4; B2/B5, B3/B4; B3/B5; B4/B5

- (c) ternary combinations of boosters selected from the
 group consisting of:
 B1/B2/B3; B1/B2/B4; B1/B2/B5; B1/B3/B4; B1/B3/B5;
 B1/B4/B5; B2/B3/B4; B2/B3/B5; B2/B4/B5; B3/B4/B5
- (d) quaternary combinations of boosters selected from the group consisting of:

 B1/B2/B3/B4; B1/B2/B3/B5; B1/B2/B4/B5;
 B1/B3/B4/B5; B2/B3/B4/B5;

and

15 (e) a combination of five groups of boosters: B1/B2/B3/B4/B5.

The preferred compositions include from about 0.001% to about 10%, by weight of the composition of a retinoid.

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The compounds included in the present invention as boosters are selected based on the ability of such compounds to pass, at a certain concentration listed in Table A, in-vitro Assays for a specific enzymes as described below under sections 2.1 through to 2.7. Such a booster is included in the present invention even if it is not explicitly mentioned herein. Put another way, if a compound inhibits or enhances sufficiently an enzyme in an assay described below, it will act in combination with a retinoid to mimic the effect on keratinocytes (skin cells) of retinoic acid, and thus it is included within the scope of the present invention.

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The term "conditioning" as used herein means prevention and treatment of dry skin, acne, photo-damaged skin, appearance of wrinkles, age spots, aged skin, increasing stratum corneum flexibility, lightening skin colour, controlling sebum excretion and generally increasing the quality of skin. The composition may be used to improve skin desquamation and epidermal differentiation.

10 The presence of the selected compounds in the inventive product substantially improves the performance of a retinoid.

The inventive compositions contain, as а preferred ingredient, a retinoid, which is selected from retinyl esters, retinol, retinal and retinoic acid, preferably 15 retinol or retinyl ester. The term "retinol" includes the following isomers of retinol: all-trans-retinol, 11-cis-retinol, 9-cis-retinol, 3,4-didehydroretinol, retinol, 3,4-didehydro-13-cis-retinol; 3,4-didehydro-11-cisretinol; 3,4-didehydro-9-cis-retinol. Preferred isomers are 20 13-cis-retinol, 3,4-didehydro-retinol, all-trans-retinol, 9-cis-retinol. Most preferred is all-trans-retinol, due to its wide commercial availability.

25 Retinyl ester is an ester of retinol. The term "retinol" has been defined above. Retinyl esters suitable for use in the present invention are C₁-C₃₀ esters of retinol, preferably C₂-C₂₀ esters, and most preferably C₂, C₃, and C₁₆ esters because they are more commonly available. Examples of retinyl esters include but are not limited to: retinyl palmitate, retinyl

retinyl acetate, retinyl propionate, formate, retinyl retinyl valerate, retinyl butyrate, isovalerate, retinyl hexanoate, retinyl heptanoate, retinyl octanoate, retinyl nonanoate, retinyl decanoate, retinyl undecandate, retinyl laurate, retinyl tridecanoate, retinyl myristate, retinyl pentadecanoate, retinyl heptadeconoate, retinyl stearate, isostearate, retinyl nonadecanoate, retinyl arachidonate, retinyl behenate, retinyl linoleate, and retinyl oleate.

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The preferred ester for use in the present invention is selected from retinyl palmitate, retinyl acetate and retinyl propionate, because these are the most commercially available and therefore the cheapest. Retinyl linoleate and retinyl oleate are also preferred due to their efficacy.

Retinol or retinyl ester is employed in the inventive composition in an amount of from about 0.001% to about 10%, preferably in an amount of from about 0.01% to about 1%, most preferably in an amount of from about 0.01% to about 0.5%.

The essential ingredient of the inventive compositions is a compound which passes in vitro Assays described below in sections 2.1 through to 2.7. A compound suitable for use in the present invention inhibits or enhances at a concentration listed in Table A an enzyme to at least a broad % listed in Table A.

Section A: Identification of Booster:

TABLE A
Booster Test Concentrations and % Inhibition/Increase

ARAT / LRAT Assay	(To identify B1 booster:	5)
Invention	Compound Concentration	% Inhibition
Broad	100 µM	> 10%
Preferred	100 μΜ	> 25%
Most Preferred	100 μΜ	> 40%
Optimum	100 μΜ	> 50%

Retinol Dehydrogenase Assay	(To identify B2 boosters	s)
Invention	Compound Concentration	% Increase
Broad	. 100 μM	> 10%
Preferred	100 μΜ	> 15%
Most Preferred	100 μΜ	> 20%
Optimum	100 μΜ	> 25%

10	Retinal Reductase Assay	(To identify B3 boosters)
	Invention	Compound Concentration	% Inhibition
	Broad	100 μΜ	> 5%
	Preferred	100 μΜ	> 10%
	Most Preferred	100 μΜ	> 20%
	Optimum	100 μΜ	> 35%

CRABPII Antagonist Assay	(To identify B4 boosters)	
Invention	Compound : Retinoic acid Ratio	% Inhibition
Broad	7000 : 1	> 25%
Preferred	7000 : 1	> 50%
Most Preferred	70 : 1	> 25%
Optimum	70 : 1	> 50%

Retinoic Acid Oxidation Assay (To identify B5 boosters)

Invention	Compound Concentration	% Inhibition
Broad	100 μΜ	> 25%
Preferred	100 μΜ	> 45%
Most Preferred	100 μΜ	> 70%
Optimum	100 μΜ	> 80%

The in vitro Microsomal Assays employed for determining the suitability of the inclusion of the compound in the inventive compositions are as follows:

1. Materials

All-trans-retinol, all-trans-retinoic acid, palmitoyl-CoA, 10 dilauroyl phosphatidyl choline, NAD, and NADPH were purchased from Sigma Chemical Company. Stock solutions of retinoids for microsomal assays were made up in HPLC grade the acetonitrile. All retinoid standard stock solutions for HPLC analysis were prepared in ethanol, stored under atmosphere of 15 N₂ at -70°C and maintained on ice under amber lighting when out of storage. Other chemicals and the inhibitors were commercially available from cosmetic material suppliers or chemical companies such as Aldrich or International Flavours 20 and Fragrances.

2. Methods

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2.1 Isolation of RPE microsomes (modified from (1))

50 frozen hemisected bovine eyecups, with the retina and aqueous humor removed were obtained from W. L. Lawson Co.,

Lincoln, NE, USA. The eyes were thawed overnight and the colored iridescent membrane was removed by peeling with forceps. Each eyecup was washed with 2x 0.5mL cold buffer (0.1M PO4 / 1mM DTT / 0.25M sucrose, pH 7) by rubbing the darkly pigmented cells with an artist's brush or a rubber 5 The cell suspension was added to the iridescent policeman. membranes and the suspension was stirred for several minutes in a beaker with a Teflon stir bar. The suspension was filtered through a coarse filter (Spectra/Por 925µ pore size polyethylene mesh) to remove large particles, and the resulting darkly colored suspension was homogenized using a Glas-Col with a motor driven Teflon homogenizer.

The cell homogenate was centrifuged for 30 min. at 20,000g 15 (Sorvaal model RC-5B centrifuge with an SS34 rotor in 2.5x10cm tubes at 14,000 RPM). The resulting supernatant was subjected to further centrifugation for 60 min. at 150,000g (Beckman model L80 Ultracentrifuge with an SW50.1 rotor in 13x51mm tubes at 40,000 RPM). The resulting pellets were dispersed into $\sim 5 \text{mL} \ 0.1 \text{M} \ \text{PO}_4$ / $5 \text{mM} \ \text{DTT}$, pH 7 buffer using a 20 Heat Systems Ultrasonics, Inc. model W185D Sonifier Cell and the resulting microsomal dispersion Disruptor, aliquoted into small tubes and stored at -70 $^{\circ}$ C. The protein concentrations of the microsomes were determined using the BioRad Dye binding assay, using BSA as a standard. 25

2.2 Isolation of rat liver microsomes (4)

Approximately 6 grams of frozen rat liver (obtained from 30 Harlan Sprague Dawley rats from Accurate Chemical and Scientific Corp.) was homogenized in 3 volumes of 0.1M tris /

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0.1M KCl / 1mM EDTA / 0.25M sucrose, pH 7.4 buffer using a Brinkmann Polytron. The resulting tissue suspension was further homogenized in the motor driven Teflon homogenizer described above. The resulting homogenate was successively centrifuged for 30 min. at 10,000g, 30 min. at 20,000g, and 15 min. at 30,000g, and the resulting supernatant was ultracentrifuged for 80 min. at 105,000g. The pellet was sonicated in ~5mL of 0.1M PO4 / 0.1mM EDTA / 5mM MgCl₂, pH 7.4 buffer as described above and stored as aliquots at -70°C. Protein concentrations were determined as described above.

2.3 Assay for ARAT and LRAT activity (To identify B1)

The procedure below was a modification of a method described in the literature (2). The following buffer was prepared and 15 stored at 4°C: 0.1M PO $_4$ / 5mM dithiothreitol, pH 7.0 (PO_4/DTT). On the day of the assay, 2mg BSA per mL of buffer was added to give a PO_4 / DTT / BSA working buffer. retinol substrate was prepared in acetonitrile and stored in amber bottles under nitrogen gas at -20°C. Solutions of 4mM Palmitoyl-CoA in working buffer (stored in aliquots) and 4mM dilauroyl phosphatidyl choline in ethanol were prepared and stored at -20°C. Inhibitors were prepared as 10mM stock solutions in H_2O , ethanol, acetonitrile or DMSO. solution was prepared using pure ethanol containing $50\mu g/mL$ 25 hexane solution butylated hydroxytoluene (BHT), and a containing $50\mu g/mL$ BHT was used for the extractions.

To a 2 dram glass vial, the following were added in order: PO_4 30 / DTT / BSA buffer to give a total volume of $500\mu L$, $5\mu L$ acyl donor (4mM palmitoyl-CoA and/or dilauroyl phosphatidyl

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choline), 5µL inhibitor or solvent blank (10mM stock or further dilutions) followed by approximately 15µg of RPE (approximately 15μ L microsomal protein of a microsomal protein aliquot). The mixture was incubated for 5 min. at 37°C to equilibrate the reaction temperature and then 5µL 1mM retinol was added. The vials were capped, vortexed for 5 seconds and incubated for 30-90 minutes at 37°C. reaction was quenched by adding 0.5mL ethanol/BHT. The retinoids were extracted by adding 3mL hexane/BHT, vortexing the tubes for several seconds several times and centrifuging 10 the tubes at low speed for 5 min. to quickly separate the layers. The upper hexane layer was removed into a clean vial, and aqueous layer re-extracted with another 3mL hexane/BHT, as described above. The hexane layers 15 combined, and the hexane evaporated by drying at 37°C under a stream of nitrogen gas on a heated aluminum block. The dried residue was stored at -20°C until HPLC analysis. The amount of retinyl palmitate and retinyl laurate was quantitated for ARAT and LRAT activity, respectively, by integration of the HPLC signal as described below. 20

Note that the incubation solution contains 40 μ M acyl donor, 100 μ M or less inhibitor, 10 μ M retinol, approximately 30 μ g/mL microsomal protein, and nearly 0.1M PO₄/ pH 7 / 5mM DTT / 2mg/mL BSA. All steps subsequent to the addition of retinol were done in the dark or under amber lights.

2.4 Assay for Retinol Dehydrogenase Activity (To identify B2)

30 The following stock solutions were prepared:

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50mM KH2PO4, pH 7.4 buffer, sterile filtered.

10mM all trans Retinol (Sigma R7632) in DMSO.

200mM Nicotinamide adenine dinucleotide phosphate, sodium salt (NADP) (Sigma N0505) in sterile water.

- 5 40mM test compound in appropriate solvent (water, buffer, ethanol, chloroform or DMSO).
 - 1:10 dilution of rat liver Microsomes in 50mM KH2PO4, pH 7.4 buffer $(4\mu g/\mu l)$.
- 10 In a two-dram glass vial with screw cap, the following were added in order:

Buffer to give a final volume of $400\mu l$

25µl diluted Microsomes (final = 100µg) - boiled Microsomes 15 were used for controls and regular Microsomes for test samples.

4μl of 200mM NADP (final = 2mM)
1μl of 40mM test compound (final = 100μM)
8μl of 10mM retinol (final = 200μM)

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The vials were incubated in a 37°C shaking water bath for 45 minutes. 500µl ice-cold ethanol was added to each vial to quench the reaction. The retinoids were extracted twice with ice cold hexane (2.7ml per extraction). Retinyl acetate (5µl of a $900\mu\text{M}$ stock) was added to each vial during the first 25 extraction as a means of monitoring the extraction efficiency in each sample. Samples were vortexed for ten seconds before gently centrifuging for five minutes at 1000rpm, 5°C in a Beckman GS-6R centrifuge. The top hexane layer containing the retinoids was removed from the aqueous layer after each 30 was two-dram vial. The extraction to a clean

evaporated off under a gentle stream of nitrogen gas. The dried residue was then stored at -20°C until HPLC analysis.

2.5 Assay for Retinal Reductase Activity (To identify B3)

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All stock solution were prepared as above with the following substitutions:

10mM all trans Retinaldehyde (Sigma R2500) in DMSO - instead 10 of retinol.

200mM, Nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt (NADPH) (Sigma N7505) in sterile water — instead of NADP.

15 In a two-dram glass vial with screw cap, add the following in order:

Buffer to give a final volume of 400µl

25µl diluted Microsomes (final = $100\mu g$) - use boiled 20 Microsomes for controls and regular Microsomes for test samples.

 $4\mu l$ of 200mM NADPH (final = 2mM)

1 μ l of 40mM test compound (final = 100 μ M)

 $3\mu l$ of 10mM retinaldehyde (final = $75\mu M$)

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Follow the same incubation and extraction procedure as detailed above.

2.6 Assay for CRABPII antagonists (To identify B4)

2.6.1. Synthesis of CRABPII

- a. System of expression
- 5 The gene CRABPII was cloned in pET 29a-c(+) plasmid (Novagen). The cloned gene was under control of strong bacteriophage T7 transcription and translation signals. The source of T7 polymerase was provided by the host cell E.coli BLR(DE3)pLysS (Novagen). The latter has a chromosomal copy of T7 polymerase under lacUV5 control, induced by the presence of IPTG.

The plasmid was transferred into E. coli BLR(DE3)pLysS cells by transformation according to the manufacturer protocol (Novagen).

b. Induction

An overnight culture of the transformed cells was diluted 1:100 into 2xYT containing 50 µg/mL kanamycin and 25µg/mL chloramphenicol. The cells grew while shaking at 37°C until the OD at 600 nm reached 0.6-0.8. Then IPTG was added to a final concentration of 1mM and the culture was incubated for an additional two hours. The cells were harvested by centrifugation at 5,000g for 10 minutes at room temperature.

25 The pellet was stored at -20°C.

2.6.2. Purification

Purification was performed according to the method described in Norris and Li, 1997.

30 a. Lysis

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The frozen pellet was thawed at RT and resuspended in 1-2 pellet volumes of freshly prepared lysis buffer (50 mM Tris-HCl, pH 8, 10%(w/v) sucrose, 1 mM EDTA, 0.05%(w/v) sodium azide, 0.5 mM DTT, 10 mM MnCl₂, 2.5 mM phenylmethylsulfonyl fluoride, 2.5 mM benzamidine, 6µg/mL DNase). The lysate was incubated for 30 mins. at room temperature. Further lysis was accomplished by sonication (six 30-sec bursts at 10,000 psi alternated with five 30-sec delay on ice). The insoluble fraction of the lysate was removed by centrifugation at 15,000 rpm 1 hour at 4°C and the supernatant is stored at -20°C.

- b. Gel filtration on Sephacryl S300
- The supernatant from step a. was loaded onto a 2.5×100 cm column of sephacryl S-300 (Pharmacia) at room temperature. 15 The elution buffer was 20 mM Tris-HCl, pH 8, 0.5mM DTT, 0.05% sodium azide (buffer A). The flow rate was were checked for ultraviolet fractions Collected 2-mL The fractions representing the peaks absorbance at 280 nm. were examined by SDS-page for the presence of CRABPII. 20
 - c. Anion-exchange chromatography
- 2 mL of gel filtration fractions containing CRABPII were loaded onto a quaternary amine anion-exchange column FPLC (Fast Protein Liquid Chromatography) type monoQ (Pharmacia). CRABPII was eluted using a gradient buffer from 100% buffer A to 30% buffer B (100 % buffer B = buffer A + 250 mM NaCl) over a 20-min period at room temperature. 1 mL-fractions were collected every minute. Once more, the presence of CRABPII was checked by SDS page. CRABPII was stored at 4°C before freeze-drying using a Micromodulyo 1.5K with vial

platform attachment (Edwards High Vacuum International). The desiccated samples were stored at room temperature until their use in the binding assay.

d. Detection of the presence of CRABPII 5 The expression and purification of CRABPII was validated using denaturing SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis on a 7-15% polyacrylamide gel (Biorad). samples were mixed with 10 µL of 2X loading buffer (100 mM 10 Tris-HCl pH6.8, 4% SDS, 0.2% BPB, 20% glycerol, 1mM DTT) and denatured by heating (2 mins. at 80°C). The samples were loaded onto the gel that was immersed in a 1X Tris-glycine buffer (Biorad) and a constant current (25 mA) was applied for 1 hour at room temperature. After Coomassie blue 15 the protein was identified according molecular weight as determined with the Benchmark pre-stained protein ladder (Gibco BRL).

A western blot was used to confirm the presence of CRABPII. 20 The proteins separated on the SDS-PAGE were transferred on an Immobilon-P transfer membrane (Millipore) using a Biorad The transfer occurred in 1X Tris-glycine buffer cassette. (Biorad) + 10% methanol. An electrical currant (60 mA) was applied for 3 hours to allow the protein to migrate through 25 the membrane. Afterwards, the membrane was blocked with 5% dry milk in 1X TBS for one hour at room temperature and probed with primary antibodies to CRABPII (1/1000 dilution of anticlonal 5-CRA-B3) in the same buffer The following day, the membrane was washed with PBS (3 \times 5 minutes) and then incubated with 1:2000 dilution 30 of the secondary antibody, peroxidase conjugated anti-mouse

antibody (ECLTM, Amersham), for 1 hour at room temperature. The membrane was washed with 1xPBS (3x5 minutes) and the protein was detected using ECL detection kit according to the manufacturer instruction (Amersham).

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The concentration of purified CRABPII was determined using BSA kit (Pierce).

2.6.3. Radioactive Binding assay

- 10 220 pmol of CRABPII was incubated in 20 mM Tris-HCl buffer pH 7.4 with 15 pmol of radioactive all trans retinoic acid (NEN) in a total volume of 70µL. For the competitive assay, another ligand in excess (6670:1, 670:1 or 70:1) was added to The reaction occurred for one hour at room the mix. temperature in the dark. In order to separate the unbound 15 all-trans retinoic acid from the bound all-trans retinoic acid, a 6kD cut-off minichromatography column (Biorad) was The storage buffer was discarded using a Microplex manifold for according to the manufacturer instruction (Pharmacia). The samples were loaded onto the column and the 20 separation occurred by gravity over a 30-min Retinoic acid ("RA") bound to CRABPII appeared in filtrate while free RA remained in the column. radioactivity of the filtrate was measured by scintillation 25 counter.
 - 2.7 Assay for NADPH dependent retinoic acid oxidation (To identify B5)
- 30 The procedure below is a modification of a method described in the literature (4). The following assay buffer was

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prepared and stored at 4°C: $0.1M\ PO_4$ / $0.1mM\ EDTA$ / $5mM\ MgCl_2$, pH 7.4. On the day of the assay, a 60mM NADPH solution in buffer was prepared. Inhibitor stocks, acidified ethanol / BHT quench solution, and hexane / BHT were prepared as described above. A working 1mM retinoic acid solution was prepared by dilution of a 15mM stock (in DMSO) with ethanol.

To a 2 dram vial, the following were added in order: assay buffer to give a final volume of 500μL, 20μL 60mM NADPH, 5μL inhibitor or solvent blank, followed by approximately 2mg of rat liver microsomal protein.

The mixture was incubated for 5 mins. at 37°C, then 5µL working 1mM retinoic acid solution was added. Incubation was continued for 60mins. at 37°C - the vials were not capped, 15 since the oxidation process required molecular O2 in addition acidified NADPH. Quenching was carried out with to ethanol/BHT and extraction was carried out with hexane/BHT as described above. Quantitation of the quickly eluting polar retinoic acid metabolites (presumed to be 4-oxo retinoic 20 acid) was carried out by integration of the HPLC signal as described below.

All steps subsequent to the addition of retinoic acid were done in the dark or under amber lights. The final incubation solution contained 2.4mM NADPH, 100µM or less inhibitor, 10µM retinoic acid, approximately 4mg/mL rat liver microsomal protein and nearly 0.1M PO₄ / 0.1mM EDTA / 5mM MgCl₂.

30 HPLC analysis of individual retinoids

Samples for retinoid quantitation by HPLC were prepared by dissolving the residue in each vial with 100µL of methanol. The solution was transferred to a 150µL glass conical tube within a 1mL shell vial, capped tightly, and placed inside a Waters 715 Autosampler. Aliquots of 60µL were injected immediately and analysed for retinoid content.

The chromatography instrumentation consisted of a Waters 600 gradient controller/pump, a Waters 996 Photodiode Array detector and a Waters 474 Scanning Fluorescence detector. Two HPLC protocols were used for retinoid analysis. For the ARAT and LRAT assay, the separation of retinol and retinol esters was performed with a Waters 3.9x300mm C18 Novapak reverse-phase analytical column and Waters Sentry NovaPak C18 guard column with an 80:20(v/v) methanol/THF isocratic mobile phase adjusted to a flow rate of lmL/min. for 10 min. The eluate was monitored for absorbance at 325nm and fluorescence at 325ex/480em.

- 20 A shorter Waters 3.9x150mm C18 Novapak reverse-phase analytical column and Waters Sentry NovaPak C18 guard column were used to separate retinoid acids and alcohols for the retinol and retinoic acid oxidation assays utilising a modification of a gradient system described by Barua (5).
- This system consisted of a 20 mins. linear gradient from 68:32(v/v) methanol/ water containing 10mM ammonium acetate to 4:1(v/v) methanol:dichloromethane followed by a 5 mins. hold at a flow rate of 1mL/min. The column eluate was monitored from 300nm to 400nm.

These protocols were selected based on their ability to retinoid acids, alcohols, pertinent resolve aldehydes, and/or esters for each assay and relative Identification of individual separation. quickness of retinoids by HPLC was based on an exact match of the retention time of unknown peaks with that of available authentic retinoid standards and UV spectra analysis (300unknown peaks against available 400nm) of retinoids.

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References

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- Retinol Esterification in Retinal Pigment Epithelium", J. Bill. Chem. 263, 8084-8090 (1988).
 - J. C. Saari & D. L. Bredberg, "ARAT & LRAT Activities of Bovine Retinal Pigment E p i t h e l i a l Microsomes", Methods Enzymol. 190, 156-163 (1990).
- 20 3 J. L. Napoli & K. R. Race, "The Biosynthesis of Retinoic Acid from Retinol by Rat Tissues in vitro", Archives Biochem. Biophys. 255, 95-101 (1987).
 - 4 R. Martini & M. Murray, "Participation of P450 3A Enzymes in Rat Hepatic Microsomal Retinoic Acid 4-Hydroxylation", Archives Biochem. Biophys. 303, 57-66 (1993).
 - 5 A. B. Barua, "Analysis of Water-Soluble Compounds: Glucuronides", Methods Enzymol. 189, 136-145 (1990).
- The boosters suitable for use in the present invention include but are not limited to the boosters listed in Tables B_1 through to B_5 below. The table below gives the booster

class (B_1 - B_5), the chemical name of the compound, and the results from the appropriate assays used to identify the booster (i.e. ARAT/LRAT for B1, retinol dehydrogenase for B_2 , retinaldehyde inhibation for B_3 , CRABP is binding for B_4 and retinoic acid oxidation inhibition for B_5 .

ARAT/LRAT Inhibitors	ors (B1)						*	
Class	Compound	*innibi cion Overall TG(-ROH/RE)	Overall TG (IC 50)	%Inhibition ARAI (10Jm)	%Inhibition ARAT (100jm)	%Inhibition LRAT (10jm)	%inhibition LRAT (100jm)	
			3.75E-05	15%	34%	0	15%	
	Crocetin		6.78E-06	198+/-12	628+/-11	108+/-10	07-/+%00	
ч	ארפרלד ההיינוסים אלים	17%			28%		25%	
Fatty Acid Amides &	Cl3 Beta-Hydroxy Acta, amide	ે ને	1					
Other Surractants Fatty Acid Amides &	Castor Oil MEA		3.25E-05		i i			
	Cocamidopropyl Betaine		`		\$C7		64 U	
Surfactants			2.84E-07		68 8		o O	
	coco hydroxyemyr imidazoline	010			13%		348	
Fatty Acid Amides 6 Other Surfactants	Cocoyl Monoethanol-						C-/+8857	
	amide)				418+/-6		7 / 1400	
Fatty Acid Amides &	Glycerol-FCA-Oleace				20%			
Statty Acid Amides &	Hexanoamide						0-/+868	
Other Surfactants	Heyanovi Sphingosine		9.99E-05		288+/-4			
Acid Amides	Samuel Louisvall		ני ני ני		35%		35%	
Fatty Acid Amides &	Hydroxyethy1-2-		3.29E-05		i (80	
Surfactants	Hydroxy-C12 Amide				25%)	
Fatty Acid Amides & Other Surfactants	Hydroxy-C16 Amide				20%			
	Lauroyl Sarcosine			•	6		0	
Other Surfactants Fatty Acid Amides &	Lidocaine				\$2T		7 + 8 - 7	
	Linoleamide-DEA (or	59%		128+/-3	438+/-3	TT%+/ T		
	Linoleoyl				c C	308+/-8	35%	
מיים ליותם ליות בייות בי	Diethanolamide/ Linoleamide-MEA (or		1.61E-05	148	33%			
Surfactants	Linoleoyl Monoethanol-				81-/+803		758+/-4	
משטישמ נייטא יידווני					2		, ,	
					648+/-15		4384/-27	
Acid Amides Surfactants		·			418+/-14		118+/-11	
Fatty Acid Amides								

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Fatty Acid Amides &	Oleyl Betaine		2.80E-05		478		
Other Surfactants Fatty Acid Amides &	Palmitamide-MEA			%	23%	12%	(N) (N) (N)
Other Surfactants Fatty Acid Amides &	Stearylhydroxyamide				10%		10%
Other Surfactants Fatty Acid Amides &	Utrecht-1	21%		43%	548	51%	488+/-6
Other Surfactants Fatty Acid Amides &	Utrecht-2		3.47E-06	428	838+/-9	51%	92%+/-3
Other Surfactants	Naringenin				33%		148
Fragrances	Allyl Alpha-Ionone			168+/-14	1+8	178+/-10	368/-7
Fragrances	Alpha-Damascone		3.35E-04	678+/-27	-/+	878+/-6	`
Fragrances	Alpha-Ionone		.27		1+%		498+/-30
Fragrances	Alpha-Methyl Ionone		٠		678		77%
Fragrances	Alpha-Terpineol				26%		25%
Fragrances	Beta-Damascone			45%	84%	52%	92%
Fragrances	Brahmanol				70%		75%
Fragrances	Damascenone			23%	708	29%	79%
Fragrances	Delta-Damascone			58%	878	4	95%
Fragrances	Dihydro Alpha-Ionone				13%		18%
Fragrances	Ethyl Saffranate				51%		49%
Fragrances	Fenchyl Alcohol				12%		4%
Fragrances	Gamma-Methyl Ionone				21%		38%
Fragrances	'Isobutyl Ionone				% 00		45%
Fragrances	Isocyclogeraniol				18%		16%
Fragrances	Isodamascone				808		928
Fragrances	Lyral		1.27E-04		76%		718
Fragrances	Santalone				238		128
Fragrances	Santanol				158		43%
Fragrances	Timberol				34%		33%
Fragrances	Tonalid				50%		33%
Fragrances	Traseolide				418		218
Miscellaneous	Coco Trimethyl-				27%	•	
	ammonium Cl-				•		20%
Miscellaneous	Urosolic Acid		1.46E-06		% ⁴ 0		Ö
Noncyclic Fragrances	Citral				0		
•							

Other Surfactants

:

					o C		c	
Noncyclic	Citronellol				30%		>	
Fragrances		,	1			7	. 01-71-868	
Noncyclic	Farnesol	ה ה	9.35E-05	238+/-18	274/-78	/ - / + % O T	77847	
Fragrances	,	ſ		6	900			
Noncyclic	Geraniol	a.,	7.83E-03	₩ T	378			
Fragrances						0 / 10 / 1	51-11376	
Noncyclic	Geranyl Geraniol			388+/-12	9-/+878	108+/-9	CT1 /+9//	
Fragrances					•		c	
Noncyclic	Linalool				%8Z		Þ	
Fragrances					0			
Noncyclic	Nonadieneal				802			
Fragrances							0,10	
Noncyclic	Pseudoionone				*ZT		9/0	
Fragrances						c	710.7	
Phospholipid	Dioctylphosphatidyl			23%	2-/+%00	9	17-14817	
	Ethanolamine							
Urea	Dimethyl	22%						
	Imidazolidinone							
100	Trear	ንጉ%						

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(B2)
Activators
Dehydrogenase
Retinol

enase		
Dehydrog	increase	increase
Retinol	21% incr	26% incr
%Increase Retinol Dehydrogenase		
Compound	Phosphatidyl Choline	Sphingomyelin
Class	Phospholipid	Phospholipid

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Retinaldehyde Reductase Inhibitors (B3)

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		Overall	% Inhibition	
Class	Compound	TG(IC 50)	Retinal Reductase	
Aldehyde	Vanillin	9.70E-03	69	
Fatty Acid	Arachidic Acid		20%	
Fatty Acid	Arachidonic Acid		49%	
Fatty Acid	Linoleic Acid	1.63E-04	628+/-2	
Fatty Acid	Linolenic Acid	1.34E-04	548+/-16	
Fatty Acid	Myristic Acid	1.72E-05	26%	
Miscellaneous	Amsacrine	6.26E-06	22%+/-8	
Miscellaneous	Carbenoxolone	3.61E-07	268+/-2	
Miscellaneous	Glycyrretinic Acid	8.64E-06	38%+/-1	
Phospholipid	Phosphatidyl ethanolamine		37%	

CRABPII Antagonists (B4)

% Inhibition	CRABPII	>50%	>50%	>50%	>20%	>20%
Overall	TG(IC 50)	6.50E-05	1.30E-04	2.91E-05	6.88E-05	
	Compound	Elaidic Acid	Hexadecanedioic Acid	12-Hydroxystearic Acid	Isostearic Acid	Linseed Oil
	Class	Fatty Acid	Fatty Acid	Fatty Acid	Fatty Acid	Fatty Acids

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Retinoic Acid Oxidation Inhibitors (B5)	on Inhibitors (B5)				
		Overall	%Inhibition	% Inhibition	
		TG(IC 50)	Retinoic	Retinoic	
Class	Compound		Acid (10µM)	Acid (100µM)	
Imidazole	Bifonazole		868	100%	
Imidazole	Climbazole	4.47E-06	808	92%	
Imidazole	Clotrimazole	•	.76%	85%	
Imidazole	Econazole		888	100%	
Imidazole	Ketoconazole	1.85E-07	848	84%	
Imidazole	Miconazole	2.78E-07	748	86%	
Fatty Acid Amides & Other	Lauryl Hydroxyethylimidazoline	4.67E-07			
Surfactants					•
Fatty Acid Amides & Other	Oleyl Hydroxyethylimidazoline	3.02E-05	548	808	
Surfactants					
Flavanoids	Quercetin	6.29E-05	408	748	
Coumarin	Coumarin				
Quinoline	(7H-Benzimidazo [2,1-a]Benz [de]-Isoquinolin-7-one	8.59E-07			
Quinoline	Hydroxyquinoline (Carbostyril)	3.64E-04			
Quinoline	Metyrapone (2-Methyl-1,2-di-3- Pyridyl-1-Propane)			478	

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SECTION B. Effects Of Booster Combinations

In order to assess the effect of combinations of booster molecules an assay is required which encompasses the effect 5 of each of the five booster classes. A single enzyme assay is not suitable for this purpose, as it will be specific only for one class of booster molecule. An assay which retinoid concentration in keratinocytes reflects necessary to relate the effects of single booster molecules 10 with combination of booster molecules. For this reason, a transglutaminase (Tgase) assay was utilised. calcium dependent enzymes that catalyse the formation of covalent cross-links in proteins. Several Tgase enzymes are membrane bound in keratinocytes which is important 15 epidermal cell maturation. This enzyme is inhibited by The higher the concentration of retinoic retinoic acid. acid, the greater the inhibition of Tgase expression. Tgase is a good marker of both keratinocyte differentiation and of the retinoid effect on keratinocytes. 20

Transglutaminase as a marker of skin differentiation

During' the process of terminal differentiation epidermis, a 15nm thick layer of protein, known as the 25 cornified envelope (CE) is formed on the inner surface of the The CE is composed of numerous distinct cell periphery. been cross-linked together proteins which have lysine isodipeptide formation of $N\Sigma - (\gamma - glutamyl)$ two different action of at least catalysed by the 30

I is expressed in abundance in the differentiated layers of the epidermis, especially the granular layer, but is absent in the undifferentiated basal epidermis. Thus TGase I is a useful marker of epidermal keratinocyte differentiation with high TGase I levels indicating a more differentiated state. An ELISA based TGase I assay, using a TGase I antibody, was used to assess the state of differentiation of the cultured keratinocytes in the examples that follow.

10

Keratinocytes (cultured as described above) were plated in 96 well plates at a density of 4,000-5,000 cells per well in After incubation for two to three days, or 200ul media. until cells are ~50% confluent, the media was changed to media containing test compounds (five replicates per test). 15 The cells were cultured for a further 96 hours after which time the media was aspirated and the plates stored at -70°C. Plates were removed from the freezer, and the cells were washed twice with 200µl of 1xPBS. The cells were incubated for one hour at room temperature (R/T) with TBS/5% BSA (wash 20 Next the TGase primary buffer, bovine serum albumin). antibody was added: 50µl of monoclonal anti-Tgase I Ab B.C. diluted 1:2000 in wash buffer. The primary antibody was incubated for 2 hours at 37°C and then rinsed 6x with wash buffer. Cells were then incubated with 50µl of secondary 25 antibody (Fab fragment, peroxidase conjugated anti-mouse IgG obtaining from Amersham) diluted 1:4,000 in wash buffer for two hours at 37°C, then rinsed three times with wash buffer. Following the rinse with washing buffer, the cells were 30 rinsed 3x with PBS. For colourimetric development, the cells

were incubated with $100\mu l$ substrate solution (4 mg ophenylenediamine and $3.3~\mu l$ $30\%~H_2O_2$ in 10ml~0.1M citrate buffer pH 5.0) for exactly five minutes, R/T, in darkness (under aluminum foil). The reaction was stopped by the addition of $50\mu l$ $4N~H_2SO_4$. The absorbance of samples was read at 492nm in a 96 well plate UV spectrophotometer. Out of the five replicates, four were treated with both antibodies, the fifth one was use as a Tgase background control. TGase levels were determined and expressed as percentage control.

10

Details of of Tgase assay:

Prior to initiating experiments, to determine the effects of combinations of booster molecules standard Tgase assay conditions were investigated. A fully validated Tgase assay was established as follows:

A. Reagents

20 Cells: Human Keratinocytes
(P2 in T75 flasks; P3 in 96
well assay plates)
Primary Antibody: TGm specific
monoclonal Ab B.C1

Biogenesis (Cat# 5560 -6006)

Neonatal Human foreskin

Secondary Ab: Peroxidase labeled antimouse Ig F(ab)2

Amersham (Cat # NA9310)

Substrate solution: For 10 ml phosphate citrate buffer 4.0 mg o-phenylenediamine 3.3 µl of 30% H₂O₂

Sigma P-7288 Sigma H-1909

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В.	Media/Buffers	3

Keratinocyte Growth Media (KGM) Clonetics (Cat# 3111)

5 Phosphate Buffered Saline; Dulbecco's without Ca/MgCl₂)

Tris Buffered Saline

10 Blocking buffer (1xTBS + 5%
dry milk)

Washing buffer (1% dry milk in TBS + 0.05% Tween 20)

Phosphate citrate buffer: 1:1
mixture of 0.2M dibasic
sodium phosphate and 0.1 M

citric acid

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4 N H₂SO₄

C. Culture ware

96-well polypropylene microtitre plate
96-well polypropylene U-bottom plate

30 T75- vent cap

D: Instrumentation/Equipment

35 Biotek Model EL 340 Microplate reader Multiprobe II

.

Life Technology (Cat # 14200-075)

BioRad (Cat #170-6404)

Sigma (Cat # P-7949)

Sigma (Cat # S-9763)

Sigma (Cat # C-1909)

Costar (Cat # 3595)

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Costar (Cat # 3794) Costar (Cat # 3376)

Bio-tek Instuments Inc. Packard

E: Cell Culture Procedure

Seeding of Keratinocytes in 96 well plates

- 5 1. A suspension of keratinocytes was prepared at a concentration of 3000 cells/200 μ l/ well in KGM medium (Used $3x10^5$ cells /12 ml media in each microtitre plate)
 - 2. 200 μ l of the keratinocyte suspension was transferred into each of the inner 60 wells only.
- $10~3.200\mu l$ of KGM media was pipetted into the outer wells (to maintain thermal equilibrium).
 - 4. Each plate was incubated at 37°C and 5% CO_2 for 3 days or until cells are ~50% confluent.

15 Treatment of keratinocytes with samples.

- 5. Stock solutions of the samples were prepared in DMSO.
- 6. The samples were diluted to desired concentration with the final assay concentration of DMSO being 0.1 %.
- 7. 20 μl of the sample was transferred into wells and 180 μl of KGM medium added to give a final assay volume of 200 μl .
 - 8. Plates were incubated at 37°C and 5% CO₂ for 72 hours.
 - 9. Media were completely removed from each well.
- 25 10. Wells were rinsed with 2x with 200 μ l of 1xPBS
 - 11. Finally they were frozen for at least 1.5 hours at -70°C .

F: Transglutaminase Assay

1. Block:

5

Incubate plates at room temperature with 200 μ l/well of blocking buffer for 1 hour.

2. Primary Antibody:

Aspirate blocking buffer. Incubated with 100 μ l/well of TGm-specific monoclonal antibody B.Cl (diluted 1:2000 in washing buffer) at 37°C for at least 2 hours.

- The primary antibody was not added in background control wells.
 - 3. Rinsed wells 6x with washing buffer.
 - 4. Secondary Antibody:

Incubated with 100 μ l/well peroxidase labeled antimouse IgF(ab)2 fragment (diluted 1:4000 in washing buffer) at 37°C for 2 hours.

- 5. Rinsed wells 3X with washing buffer (added 200 μ l) and aspirated after each rinse.
- 6. Rinsed wells 3X with PBS w/o Tween.
- 7. Incubated with 100 μ l/well substrate solution at room temperature for exactly 5 minutes.
 - 8. Stopped reaction with 50 μ l/well 4N H₂SO₄.
 - 9. Read absorbance at 492 nm in the Bio-tek plate reader.
- 25 I. Optimization Studies

a. Time Course of Transglutaminase Production

A time course experiment was conducted to determine the optimal incubation time for transglutaminase production in keratinocytes grown in 96-well plates (4000)

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cells/well). This time course study was conducted with multiple variables including dose response analyses of retinoic acid and retinol as well as incubation in the presence of 1.2 mM CaCl2. Although the transglutaminase production in the control cells (0.1% DMSO) was altered, both retinoic acid and retinol exhibited a dose dependent inhibition of transglutaminase production over The most pronounced the five day incubation period. retinoid effect was observed on day 2 and day 3. maximal inhibition was observed on day 2 with the transglutaminase production being inhibited by 85% 55% in the presence of the highest concentration (1 μ M) of retinoic acid and retinol respectively. experiment was also conducted with varying cell density (3000 cells/well and 5000 cells/well) and comparable results were observed.

1.1

B: DMSO Sensitivity

Various concentrations of DMSO ranging from 0-2% were tested for the effect on transglutaminase production in keratinocytes. The assay was sensitive to DMSO concentration with significant inhibition of activity, above 0.5% DMSO. Hence, a final assay concentration of 0.1% was selected for subsequent sample concentration studies.

C: Dose Response Curves: Retinoic Acid and Retinol

Based on the data, day 3 was selected as the optimal time and 0.1%DMSO was selected as the concentration to be used for further testing. An additional dose

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response experiment was carried out with retinoic acid and retinol in the presence of 0.1% DMSO, with the transglutaminase production being assayed on day 3. A good dose response was observed for Tgase inhibition by 10-7M retinol gave retinoic acid and retinol. linear the Tgase in inhibition of of concentration this concentration. Therefore, the evaluate chosen to was retinol combinations.

D: Final conditions used to test boosters or combination of boosters

Days of incubation of keratinocytes with

retinol and boosters - 3 days
Final DMSO concentration
Retinol concentration
Booster concentrations - 10-7M (0.1µM)
- 10 mM to 0.1 nM

Using the above conditions, dose response for all the different boosters (B1-B5) were tested to identify the best concentration of booster to test in combinations.

Transglutaminase levels were determined and expressed in the 25 Tables B1 through B5 either as:

- (i) % (booster + retinol inhibition / control inhibition) % (ROH inhibition / control inhibition), which measures the added effect of booster + retinol induced TGase inhibition over retinol alone, or
- (ii) as an IC50 value when the inhibitory effect of multiple booster concentrations was examined this provides the concentration of booster which, in combination with a

constant retinol concentration of 10^{-7} M, inhibits TGase by 50%.

Booster combinations and booster ratios:

5

It has been discovered surprisingly that certain compounds increase the endogenous levels of retinoic acid formation from retinol or retinyl esters by different mechanisms. These compounds are collectively called here as "retinoid 10 boosters". These include: inhibitors of ARAT/LRAT (B1 boosters), inhibitors of retinaldehyde reductase (B3 boosters), inhibitors of retinoic acid binding to CRABP-2 (B4 boosters) and inhibitors of retinoic acid oxidation catalysed by cytochrome P450 enzymes (B5 boosters), certain other compounds which enhance or activate retinol 15 dehydrogenase (B2 boosters). These boosters are coded as groups B1 through to B5, as seen in chart 1 herein above.

The boosters alone or in combination with each other,

20 potentiate the action of a retinoid by increasing the amount
of retinol available for conversion to retinoic acid and
inhibiting the degradation of retinoic acid. The boosters
act in conjunction with a retinoid (e.g. retinol, retinyl
ester, retinal, retinoic acid) the latter being present

25 endogenously in the skin. The preferred compositions,
however, include a retinoid in the composition, co-present
with a booster, to optimise performance.

The present invention includes, in part, a second composition containing from about 0.0001% to about 50%,

preferably from 0.001% to 10%, most preferably from 0.001% to 5% by weight of the composition of at least one booster compound, or a combination of binary, tertiary, quaternary or 5 booster combinations. The combined concentration of the booster combinations of 0.001% to 5% in specified ratios as shown below, inhibit transglutaminase in an in vitro transglutaminase assay to more than 50%, and a cosmetically acceptable vehicle.

- The boosters included in the inventive compositions are selected from the group consisting of:
 - a. Two boosters, wherein both are selected from the group consisting of B2, B3 and B4;
- b. Binary combinations of boosters selected from the group consisting of B1/B2; B1/B3, B1/B4; B1/B5; B2/B3, B2/B4; B2/B5; B3/B4, B3/B5; B4/B5
 - c. Ternary combinations of boosters selected from the group consisting of B1/B2/B3;B1/B2/B4;B1/B2/B5;
 B1/B3/B4;B1/B3/B5; B1/B4/B5; B2/B3/B4; B2/B3/B5;
- 20 B2/B4/B5;B3/B4/B5
 - d. Quaternary combinations of boosters selected from the group consisting of B1/B2/B3/B4; B1/B2/B3/B5; B1/B2/B4/B5; B1/B3/B4/B5; B2/B3/B4/B5; and
 - e. A combination of five groups of boosters B1/B2/B3/B4/B5.

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Booster to booster ratios:

The boosters of different classes (B1 to B5) in combinations

30 as shown above have an optimal concentration of between

0.001% to 5% in a cosmetic product at specific ratios as

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shown below for inhibition of Tgase activity to at least below 50%:

Invention	Ratios of boosters to boosters	Concentrations
5 Broad Preferred	1: 10,000 to 10,000:1 1: 1000 to 1000:1 ced 1:100 to 100:1	100 mM to 1 nM 10 mM to 10 nM 1 mM to 100 nM
Optimum	1:10 to 10:1	0.1 mM to 1 μM
Most prefer	red 1:100 to 100:1	1 mM to 10

Retinoid to booster ratios:

The preferred composition includes a retinoid (e.g.retinol, retinyl ester, and retinaldehyde) in the composition, copresent with a booster or a combination of the boosters, to optimise performance.

For optimum performance, the concentration of retinoid to booster should be present in the composition in ratios as given below:

	Invention	Ratios of boosters to retinoids	Concentrations
25	Broad	10,000:1 to 1:10,000	100 mM- 1 nM booster; 0.001-10% retinoids
	Preferred	1000:1 to 1:1000	10 mM-10 nM booster; 0.001-10% retinoid
	Most preferred	100:1 to 1:100	1 mM-100 nM booster; 0.01-1% retinoid
30			

Concentrations of individual boosters used in the examples:

35 Since the objective is to establish synergistic inhibition of transglutaminase expression by combinations of the active compounds with retinol, it was essential to determine the dose response profiles (IC₂₀ and IC₅₀ values) of the active compounds, when tested individually in the presence of

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The detailed dose response of boosters belonging retinol. to B2-B4 is given in the tables following the IC50 and IC 20 This data was used to identify an appropriate table below. inhibitory concentration of each sub-maximal compound, to eventually make it possible to putative synergistic effects of the mixtures of the active compounds in the presence of retinol. The data in the following table represents the IC50 and IC20 (80% of control) values and the concentrations used when testing synergies with combinations of boosters.

In order to demonstrate synergy of two compounds, it is essential to select concentrations to test that are at most a compound concentration that in other words, individually boosts the retinol inhibition of 15 Two such compounds should have expression by 20%. 40%. additive inhibition of Using this strategy to leaves a window of 40-100% for determine concentrations inhibition for detecting synergy of the further 20 compounds under examination.

A more challenging concentration criterion would be selecting concentrations of compounds which alone showed no inhibition effect, but in combination show inhibition. In this study however, we chose an even more challenging criteria. We selected concentrations of compounds that were 10 to 1000 fold lower than the minimally effective Tgase inhibiting concentration. Identification of synergistic combinations using such very low concentrations would mean

that the most effective synergistic combinations were identified.

Booster	Compound Name		T	Con. Used for
Class	Compound Name	IC50	IC20	synergy
Class				(binary,
				tertiary,
			(quaternary)
B1	LinoleoylMonoethanolamide	1.61E-05	1.48E-05	1E-05 to 1E-09
	(LAMEA)	1.012 00	1.105 03	12 03 00 11 05
	Palmitamide Monoethanolamide	ИД	ND	1E-06 to 1E-10
	Oleyl Betaine	2.80E-05	1.08E-05	1E-05 to 1E-8
	Naringenin	ND	ND	1E-05 to 1E-09
	Echinacea	ND	ND	1E-05 to 1E-09
	Dimethyl imidazolinone	ND	ND	1E-05 to 1E-09
	Melinamide	ND	ND	1E-05 to 1E-09
	Geranyl geraniol	ND	ND	1E-05 to 1E-09
	Farnesol	9.35E-05	7.82E-05	1E-06 to 1E-09
	Geraniol	7.83E-03	4.72E-03	1E-03 to 1E-07
	α-Damascone	3.35E-04	1.69E-04	1E-04 to 1E-08
	α -Ionone	9.27E-04	1.42E-04	1E-04 to 1E-08
	Castor oil Methyl Ester Acid	3.25E-05	9.38-E06	1E-06 to 1E-09
ĺ	(MEA)			00 00 10 09
	Ursolic Acid	1.46E-06	5.94-E07	1E-06 to 1E-09
	Utrecht-2	3.47-E06	3.30-E06	1E-06 to 1E-09
	Cocoyl	2.84E-07	9.21E-08	1E-08 to 1E-11
	hydroxyethylimidazoline			
	Acetyl sphingosine (C2	6.78E-06	5.15E-06	1E-06 to 1E-09
	Ceramide)		·	
	Hexanoyl sphingosine (C6	9.99E-05	6.94E-05	1E-05 to 1E-09
	Ceramide)		;	
	Crocetin	3.75E-05	2.52E-05	1E-05 to 1E-09
	Lyrial	1.27E-04	4.00E-05	1E-05 to 1E-09
	N-Hydroxyethyl-2-	3.29E-05	2.40E-05	1E-05 to 1E-09
	hydroxydodecyl amide			
B2	Phosphatidyl Choline	ND	· ND	1E-05 to 1E-09
	Sphingomyelin	ND	ND	1E-05 to 1E-09
	TCC	9.64E-07	6.18-E07	1E-07 to 1E-10
	1,2-dioctanoyl-sn-glycero-3-	ND	ND	1E-05 to 1E-09
	phosphoethanolamide			
B3	Amsacrine-HCl	6.26E-06	3.30E-06	1E-06 to 1E-09
	Carbenoxolone	3.61E-07	2.00E-07	1E-07 to 1E-10
	Glycyrrhetinic Acid	8.64E-06	5.96E-06	1E-06to 1E-09
	Linoleic Acid	1.63E-04	8.95E-05	1E-05 to 1E-09
	Linolenic Acid	1.34E-04	1.21E-04	1E-05 to 1E-09
	Arachidonic Acid (Na+ salt)	ND	ND	1E-05 to 1E-09
	Myristic Acid	1.72E-05	1.05E-05	1E-05 to 1E-09
	Vanilin	9.70E-03	8.47E-03	1E-03 to 1E-06
В4	Hexadecanedioic acid	1.30E-04	8.40E-05	1E-05 to 1E-09
	12-Hydroxystearic acid	2.91E-05	1.45E-05	1E-05 to 1E-09
	Elaidic acid	6.50E-05	5.88E-05	1E-05 to 1E-09
	Linseed oil	ND	ND '	1E-05 to 1E-09
	Isostearic acid	6.88E-05	6.23E-05	1E-05 to 1E-09
	2-Hydroxystearic acid	ND	ND	1E-05 to 1E-09
B5	Climbazole	4.47E-06	2.45E-07	1E-07 to 1E-10

Clotrimazole	ND	ND	1E-05 to 1E-09
Miconazole	2.78E-07	8.42E-08	1E-08 to 1E-11
	ND	ND	1E-05 to 1E-09
Coumarin	1.85E-07	5.52E-08	1E-08 to 1E-11
Ketoconazole			1E-05 to 1E-09
3,4,-Dihydro-2(1H)-	ND	ND	TE-03 (O TE-09
quinolinone (Hydrocarbostyril)			
2-	3.64E-04	1.70E-04	1E-04 to 1E-08
Hydroxyquinoline(Carbostyril)			
Amino Benzotriazole	ND	ND	1E-05 to 1E-09
	4.67E-07	2.69E-07	1E-07 to 1E-10
Lauryl	1		
hydroxyethylimidazoline	C DOE OF	5.11E-05	1E-05 to 1E-09
Quercetin	6.29E-05		1E-06 to 1E-09
Oleoyl hydroxyethlimidazoline	3.02E-05	5.65E-06	
7H-Benzimidazo[2,1-	8.59E-07	4.69E-07	1E-07 to 1E-09
a]Benz[de]-isoquinolin-7-one			<u> </u>

ND: Not determined or a clear dose response was not observed. For synergies, a wide range of concentration (4 orders of magnitude 10-5 to 10-9M) was tested.

Dose response for boosters class B2 to B4

The following tables include the data on the dose response 10 of boosters belonging to class B2 to B4. Concentration of boosters are given in Molar; mean Tgase level and Standard deviation of 4 replicates is expressed as % of control (0.1% DMSO and 10-7M retinol). Higher numbers (close to 100 or above 100) indicate no inhibition of Tgase. The lower the 15 the inhibitor is at potent the more number, The IC50 and IC20 values were calculated concentration. from this dose response table and expressed in the above table.

B2 class boosters:

Phosphatidyl choline (B2)

Concentration	Tgase levels (Mean)	Tgase (SD)
4.4E-05	90.9	0.01
1.47E-05	120.3	10.6
4.89E-06	70.1	11.4
1.63E-06	98.8	0.00
5.43E-07	86.7	6.19
1.8E-07	75.9	20.5
6.0E-08	87.8	3.9
1.2E-08	159	42.3
2.4E-09	85.5	0.39

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Sphingomyelin (B2)

Concentration	Tgase levels	Tgase (SD)
	(Mean)	
3.0E-05	45	3.21
1.0E-05	77.8	25.5
3.33E-06	76.4	7.55
1.1E-06	98.8	0.00
3.73E-07	91.6	14.9
1.23E-07	70.0	3.63
4.10E-08	74.6	4.19
8.2E-08	115.2	1.02
1.65E-09	68.4	2.03
3.29E-10	69.2	2.1

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TCC (B2)

Concentration	Tgase levels (Mean)	Tgase (SD)
1.14E-03	36.3	4.6
3.8E-04	3.8	0.96
3.31.23E-04	-3.2	0.91
4.22E-05	-11.2	0
1.41E-06	3	4.88
4.69E-07	15.9	3.52
6.26E-08	18.9	3.12
1.25E-08	100.2	23.3
6.9E-09	77.6	21.2
1.0E-09	54.4	11.23

1,2 dioctanoyl-sn-glycero-3-phopshoethanolamide (B2)

Concentration	Tgase levels (Mean)	Tgase (SD)
1.6E-04	58.1	2.08
5.33E-05	95.4	21.3
1.78E-05	104	4.01
5.93E-06	129	0.0
1.98E-06	110	8.74
6.58E-07	92.8	15.78
2.19E-09	88.6	12.3
4.39E-08	127.3	3.39
8.78E-09	119	21.1
1.79E-9	82	15.6

B3 Class boosters

Amscrine B3

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Concentration	Tgase levels (Mean)	Tgase (SD)
3.0E-05	-10	3.29
1.0E-05	1.8	7.45
3.33E-06	64	4.2
1.1E-06	84	0
3.73E-07	109	
1.23E-07	65	6.2
4.10E-08	110	15.8
8.2E-08	131	10.5
1.65E-09		27
3.29E-10	113	18
2.4711 10	92	8.9

Carbenoxolone (B3)

Concentration	Tgase levels (Mean)	Tgase (SD)
3.0E-06	-7.1	0
1.0E-06	27.3	1.15
3.33E-07	51.7	1.13
1.1E-07	158	0
3.73E-08	126	
1.23E-08	81	4.67
4.10E-09	135	
8.2E-10	112	6.88
1.65E-10	77.8	
3.29E-11	64	10.6
	1 04	49

Glyrrhetinic acid (B3)

Concentration	Tgase levels (Mean)	Tgase (SD)
	-0.3	3.9
3.0E-04	0.7	3.55
1.0E-05	2.5	2.1
3.33E-05	96.4	0.00
1.1E-06	120	33.2
3.73E-06	112	38
1.23E-07		11
4.10E-07	93	108
8.2E-08	225	11
1.65E-08	103	6.2
3.29E-9	100	0.2

Linoleic acid (B3)

Concentration	Tgase levels (Mean)	Tgase (SD)
0 OF 03	-6	3.06
9.0E-03	0.1	2.01
3.0E-03	-16.4	16.3
1E-03	4.4	0
1.1E-04	79.2	0
3.73E-04	62.6	6.2
1.23E-05		3.69
4.10E-05	76.8	44.2
8.2E-06	146	20.2
1.65E-07	106	
3.29E-07	60.2	2.3

Linolenic acid (B3)

Concentration	Tgase levels (Mean)	Tgase (SD)
9.0E-03	-11	8.7
3.0E-03	-5.7	0.74
1E-03	-7.5	7.8
1.1E-04	-23	0
3.73E-04	68	0.57
1.23E-05	94.9	17.2
4.10E-05	65.9	0.03
8.2E-06	119	1.6
1.65E-07	77	8.5
3.29E-07	98	7.0

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Myristic acid (B3)

Concentration	Tgase levels	Tgase (SD)
	(Mean)	
1E-03	-2	4.1
1.1E-04	· -8	2.3
3.73E-04	-6	1.16
1.23E-05		
4.10E-05	75.1	1.06
8.2E-06	74.2	10.0
1.65E-07	88.9	8.4
3.29E-07	101	4.47
5.0E-08		•
1.1E-08		

Vanillin (B3)

Concentration	Tgase levels	Tgase (SD)
	(Mean)	
1.4E-02	21.5	24.2
4.8E-03	93.8	1.7
1E-03	124	15.6
1.1E-04		
3.73E-04	101	14.3
1.23E-05	82	14.6
4.10E-05	98	2.4
8.2E-06	109	22
1.65E-07	80	4
3.29E-07	93 41	

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B4 Class boosters

Hexadecanedioic acid (B4)

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Concentration	Tgase levels (Mean)	Tgase (SD)	
1E-03	·		
1.1E-04	14.2	2.7	
3.73E-04	43.4	8.4	
1.23E-05	130	0	
4.10E-05	105	14	
8.2E-06	114	12	
1.65E-07	95	1.9	
3.29E-07			
5.0E-08	74	6.7	
1.1E-08	70	10.4	

12-hydroxysteric acid (B4)

Concentration	Tgase levels (Mean)	Tgase (SD)
3.73E-04		
1.23E-05	-5.2	2.3
4.10E-05	32.4	5.3
8.2E-06	97.6	0
1.65E-07	90.2	11
3.29E-07	82	28
5.0E-08	81	3.8
1.1E-08	98	24
2.0E-08	118	28
4.3E-09	71	2.3

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Elaidic acid (B4)

Concentration	Tgase levels	Tgase (SD)	
	(Mean)		
1E-03	12.8	12.1	
1.1E-04	8	0.45	
3.73E-04	13.8	1.92	
1.23E-05	80.9	0	
4.10E-05	58.2	8.8	
8.2E-06			
1.65E-07	. 58	0.13	
3.29E-07	69	· 44	
5.0E-08	50.5	3.8	
1.1E-08			

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Linseed Oil (B4)

Concentration	Tgase levels	Tgase (SD)	
	(Mean)		
1E-04	138	15	
3.73E-05	145	2.5	
1.23E-05	88	12	
4.10E-06	113	0	
8.2E-06	113	13	
1.65E-07	96	18	
3.29E-07	106	10	
5.0E-08	134	22	
1.1E-09	83	13	
9.9E-10	73	15	

Isosteric acid (B4)

Concentration	Tgase levels (Mean)	Tgase (SD)	
1E-03	-8.6	3.4	
1.1E-04	1.2	3.0	
3.73E-04	-5.3	1.1	
1.23E-05	80	00	
4.10E-05	67	7.9	
8.2E-06	103	12.3	
1.65E-07	95	5.5	
3.29E-07	123	0.5	
5.0E-08	78	12.2	
1.1E-08	78	29	

Concentration	Tgase levels (Mean)	Tgase (SD)
9.1E-04	46.6	6.2
3.73E-04	69.3	8.3
1.23E-04	51	8.8
3.10E-05	96.0	0.0
1.2E-05	105	30
3.65E-06	63 8.0	
1.29E-06	80	4.7
2.0E-07	142	34
5.1E-08	64	20
1.0E-08	5817	

Synergy of Tgase inhibition with binary combinations of boosters

To investigate synergistic inhibition of Tgase expression by 10 2 different classes of boosters of combinations retinol, selected combinations of compounds were tested at concentrations given in the above table. I The concentrations tested were one log order of magnitude less than the concentration required for minimal inhibition of 15 activity (i.e. IC_{20}). The compounds were tested alone and in combination and the % inhibition of Tgase is given for each compound and the combination.

- The following examples give the synergistic combinations in all possible binary combinations (B1/B2; B1/B3, B1/B4; B1/B5; B2/B3, B2/B4; B2/B5; B3/B4, B3/B5; B4/B5). When the % inhibition of the combination is more than the inhibition of each compound added together, it indicates synergy (i.e.
- 25 Inhibition by combination is greater than inhibition by

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compound 1 + compound 2). All the binary combination examples given in the following table synergistically inhibited Tgase.

Binary	Compound 1	Compound 2	TG as	TG as	TG % C Combination
combinations	1	i	% C Compd	7	Compination
			1	Compd 2	
B1/B2	Dimethyl	Phosphatidylcholine	99	97	84
	imidazolidinone				
B1/B2	Alpha-demascone	Phospahtidylcholine	95	97	86
B1/B2	Hexanoyl sphingosine	Phospahtidylcholine	109	97	86
B1/B2	Alpha-ionone	Sphingomyelin	101	98	76
B1/B2	1,2 dioctanoyl-sn-	Phosphatidyl choline	106	98	78
	glycero-3-				
	phosphoethanolamide				
B1/B2	Alpha-demascone	Sphingomyelin	95	84	67
					
B1/B3	1,2 dioctanoyl-sn-	Amsacrine	123	134	75
	glycero-3-				
	phosphoethanolamide		100	160	<u> </u>
B1/B3	1,2 dioctanoyl-sn-	Carbenoxelone	123	164	96
	glycero-3-		}		
	phosphoethanolamide			 	
B1/B3	Caster oil MEA	Carbenoxelone	96	164	67
B1/B3	Utrecht-2	Amsacrine	102	98	86 91
B1/B3	Utrecht-2	Carbenoxelone	102	164	78
B1/B3	Hexanoyl sphingosine	Carbenoxelone	122	164	
B1/B3	Lyral	Carbenoxelone	120	164	82
B1/B3	Castor oil MEA	Carbenoxelone	110	164	78
B1/B3	Hexanoyl sphingosine	Amsacrine	122	134	92
B1/B3	Hexanoyl sphingosine	Eliadic acid	122	144	85
B1/B3	Alpha ionone	Amsacrine	101	134	78
B1/B3	1,2 dioctanoyl-sn-	Glyccyrrhetinic acid	95	92	69
	glycero-3-			1	
	phosphoethanolamide			 	ļ
B1/B4	Naringenin	2- hydroxy steric acid	95	112	78
B1/B4	Hexanoyl sphingosine	2- hydroxy steric acid	99.3	112	77
B1/B4 -	Lyral	Hexadecanoic acid	120	95	69
B1/B4	Castor oil MEA	Hexadecanedioic acid	110	125	82
B1/B4	Hexanoyl sphingosine	Isostearic acid	122	146	93
B1/B4	Oleoyl betaine	Hexadecanedioic acid	99.5	125	80
51/5.	Ozeogr Details		1		
B1/B5	Hexanoyl sphingosine	Cocoyl	99	102	68
•		hydorxyethylimidazolin			
		e		1	
B1/B5	Farnesol	Ketokonazole	98	111	84
B1/B5	Hexanoyl sphingosine	Miconazole	99	101	56
B1/B5	Hexanoyl sphingosine	Ketoconazole	99	99	65
B1/B5	Hexanoyl sphingosine	Lauryl	99	98	51
	1	hydroxyethylimiazoline			l
B1/B5	Utrecht-2	Amino benzotriazole	122	105	83
B1/B5	Hexanoyl sphingosine	3,4-dihydro-2	122	102	89
		quinolinone ,			<u> </u>
B1/B5	Hexanoyl sphingosine	Amino benzotriazole	122	126	85
B1/B5	Castor oil MEA	Lauryl	110	98	56
		hydroxyethylimiazoline		J	
B1/B5	Hexanoyl sphingosine	Climbazole	122	98	83
B1/B5	Hexanoyl sphingosine	Miconazole	122	99	78
B1/B5	Hexanoyl sphingosine	Ketoconazole	122	110	90

B1/B5	Oleoyl beatine	ketoconazole	96	116	81
B1/B5	Utrecht-2	Lauryl	122	98	57
		hydroxyethylimiazoline			
B1/B5	Alpha-demascone	Oleoyl	112	73	76
	-	hydroxyethylimiazoline		1 1	
B1/B5	Alpha-ionone	Lauryl	101	98	49
_		hydroxyethylimiazoline		1	
B1/B5	Alpha-ionone	Oleoyl	101	73	75
		hydroxyethylimiazoline			
B2/B3	Phosphatidyl choline	Glycyrrhetinic acid	98	92	73
				1	
B2/B4	Phosphatidyl choline	2-hydroxy steric acid	98	82	70
B2/B5	Phosphatidyl choline	Climbazole	98	102	82
B2/B5	Phosphatidyl choline	Miconazole	98	111	92
B2/B5		Ketoconazole	98		89
	Phosphatidyl choline	Lauryl	98	101	82
B2/B5	Phosphatidyl choline	hydorxyimidazoline	98	106	82
		nydorxymmuazorine	 	 	
B3/B4	Amscarine	2-hydroxy steric acid	102	82	75
B3/B4	Myristic acid	2-hydroxy steric acid	110	82	78
			l		
B3/B5	Amscarine	Aminobenzotriazole	102	98	84 .
B3/B5	Amscarine	Dimethyl imidazoline	102	112	94
B3/B5	Myristic acid	Climbazole	110	102	82
B4/B5	Linseed oil	Lauryl hydroxyethyl imidazoline	98	73	57
B4/B5	2-hydroxystearic acid	Ketaconazole	92	109	77
B4/B5	Linseed oil	Oleovi	98	92	75
,	22500	hydorxyethylimdazoline	~~	"	
B4/B5	2-hydroxystearic acid	Coumarin	92	96	70
				 	

Synergy of Tgase inhibition with tertiary combinations of boosters

To investigate synergistic inhibition of Tgase expression by combinations of 3 different classes of boosters with retinol, selected combinations of compounds were tested. 10 The concentrations tested were one log order of magnitude less than the concentration required for minimal inhibition of Tgase activity (i.e. IC₂₀). The compounds were tested alone and in combination and the % inhibition of Tgase is given for each compound and the combination. The following examples give the synergistic combinations in all possible 15 tertiary combinations (B1/B2/B3;B1/B2/B4;B1/B2/B5;

B1/B3/B4;B1/B3/B5; B1/B4/B5; B2/B3/B4; B2/B3/B5; B2/B4/B5;B3/B4/B5). The % inhibition of the combination is more than the inhibition of each compound added together, which indicates synergy (i.e. Inhibition by combination is greater than inhibition by compound 1 + compound 2 + compound 3). All the examples of teritiary combinations of boosters given in the following table synergistically inhibited Tgase in the presence of 10-7M retinol.

Compound 1	Compound 2	Compound 3	TG as C Compd		TG as C Compd		TG as C Compd		TG as % C Combo	
B1/B2/B3 combinations										. .
Phosphatidyl Choline	Glycyrrhetinic Acid	Castor oil Methyl Ester Acid (MEA)		88		91		85		53 52
Phosphatidyl Choline	Glycyrrhetinic Acid	Echinacea		88		91		119		52
Phosphatidyl Choline	Glycyrrhetinic Acid	Naringenin		88		91		94		
Phosphatidyl Choline		Acetyl sphingosine (C2 Ceramide)		88		91		99		58
Phosphatidyl Choline		Farnesol		88		91		118		49
1,2-dioctanoyl-sn-	Glycyrrhetinic Acid	a-Damascone		83	L	9:	L '	89)	58
glycero-3- phosphoethanolamide 1,2-dioctanoyl-sn- glycero-3-	Phosphatidyl Choline	Naringenin		.81	ı .	81	3	94	ļ	66
phosphoethanolamide 1,2-dioctanoyl-sn-	Amsacrine-HCl	Linoleoyl Monoethanolamide		8	1	7	9	12	7	60
glycero-3- phosphoethanolamide 1,2-dioctanoyl-sn- glycero-3-	Amsacrine-HCl	(LAMEA) Palmitamide Monoethanolamide		8	1	7	9	9	5	63
phosphoethanolamide 1,2-dioctanoyl-sn- glycero-3-	Glycyrrhetinic Acid	a-Damascone		8	1	9	1	8	9	58
phosphoethanolamide 1,2-dioctanoyl-sn- glycero-3-	Glycyrrhetinic Acid	Naringenin		8	1	9	1	9	4	75
phosphoethanolamide 1,2-dioctanoyl-sn- glycero-3-	Glycyrrhetinic Acid	Echinacea		8	11	ç)1	11	9	77
phosphoethanolamide 1,2-dioctanoyl-sn- glycero-3-		Dimethyl imidazolinone		8	31	9	91	8	7	67
phosphoethanolamide Castor oil Methyl Ester Acid (MEA)		Phosphatidyl Choline	<u>.</u> .	8	35	9	95	8	8	63

B1/B2/B4	
Combinations	:

B1/B2/B5		•				
Combinations:	alimbarala	Echinacea	88	84	119	75
Phosphatidyl Choline						
Phosphatidyl Choline		Naringenin	88	84	94	83
Phosphatidyl Choline	Climbazole	Geraniol	88	84	105	. 76
Phosphatidyl Choline	Climbazole	Farnesol	88	84	118	82
Phosphatidyl Choline	Climbazole	Acetyl sphingosine (C2 Ceramide)	88	84	99	82
Phosphatidyl Choline	Miconazole	a-Ionone	88	92	88	70
Phosphatidyl Choline	Miconazole	Castor oil Methyl Ester Acid (MEA)	88	92	85	72
B1/B3/B4						
Combinations: Amsacrine-HCl	Dimethyl imidazolinone	Elaidic acid	79	87	93	0
D-Ionone	Amsacrine-HCl	12-Hydroxystearic	68	79	95	62
		acid	0.7	0.0	124	81
Lyrial	Hexadecanedioic acid	Vanillin	97	90	134	9.7
Hexanoyl sphingosine (C6 Ceramide)	Isostearic acid	Glycyrrhetinic Acid	104	87	91	58
•						
B1/B3/B5						
Combinations:						
Amsacrine-HCl	Dimethyl	2-	79	87	95	32
	imidazolinone	Hydroxyquinoline(C arbostyril)				
Amsacrine-HCl	Dimethyl	Lauryl	79	87	52	-13
	imidazolinone	hydroxyethylimidaz				
	D:	oline Overantia	79	87	92	-24
Amsacrine-HCl	Dimethyl imidazolinone	Quercetin	19	07	32	41
Amsacrine-HCl	Dimethyl	Oleoyl	79	87	76	39
	imidazolinone	hydroxyethlimidazo				
	Dimathul	line 7H-	79	87	94	32
Amsacrine-HCl	Dimethyl imidazolinone	Benzimidazo[2,1-	19	0 /	34	32
	INLIGIZOTITOTIC	a]Benz[de]-				
		isoquinolin-7-one				
Amsacrine-HCl	Dimethyl imidazolinone	Coumarin	79	87	80	30
Hexanoyl	Carbenoxolone	Oleovl	104	88	76	64
sphingosine (C6		hydroxyethlimidazo				
Ceramide)	!!	line	104	00	104	62
Hexanoyl	3,4,-Dihydro-	Vanillin	104	90	134	02
sphingosine (C6 Ceramide)	2(1H)- quinolinone(Hyd					
CCI and ac	rocarbostyril)					
Amsacrine-HCl	Amino	Echinacea		105	119	48
	Benzotriazole Amino	Cohingomuslin	104	105	60	69
Hexanoyl sphingosine (C6	Benzotriazole	Sphingomyelin	104	103	00	0,5
Ceramide)						
Amsacrine-HCl	Amino	Acetyl sphingosine	79	105	99	7
m ~	Benzotriazole Amsacrine-HCl	(C2 Ceramide) 7H-	68	79	94	54
□-Ionone	Musacrine-HCI	H- Benzimidazo[2,1-	00	13	24	٠.
		a]Benz[de]-				
		isoquinolin-7-one				

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Utrecht-2	Carbenoxolone	Quercetin	76	88	92	74
Utrecht-2		Oleoyl	76	88	76	69
Utrecht-2	Carbenoxolone	line 7H- Benzimidazo[2,1- a]Benz[de]- icogninelin-7-one	76	88	94	73
Utrecht-2	Carbenoxolone	isoquinolin-7-one 3,4,-Dihydro- 2(1H)- quinolinone(Hydroc arbostyril)	76	88	90	70
Myristic Acid	Climbazole	Geraniol	79	84	105	74
Myristic Acid	Climbazole	□-Damascone	79	84	89	73
Myristic Acid	Climbazole	Acetyl sphingosine (C2 Ceramide)	79	84	99	70
Oleyl Betaine	Ketoconazole	Carbenoxolone	62	85	88	78
Oleyl Betaine	Ketoconazole	Glycyrrhetinic Acid	62	85	91	71
Oleyl Betaine	Ketoconazole	Linoleic Acid	62	85	11	83
Oleyl Betaine	Ketoconazole	Linolenic Acid	62	85	208	80
Hexanoyl sphingosine (C6 Ceramide)	3,4,-Dihydro- 2(1H)- quinolinone(Hyd rocarbostyril)	Vanillin	104	90	134	62
	Hydroxyquinoline	Castor oil Methyl	93	95	85	75
	arbostyril) Hydroxyquinoline	Ester Acid (MEA) Naringenin	93	95	94	86
(C	arbostyril) Hydroxyquinoline	a-Damascone	93	95	89	80
	arbostyril) Hydroxyquinoline	Farnesol	. 93	95	118	82
Elaidic acid 2-	arbostyril) Hydroxyquinoline arbostyril)	Crocetin	93	95	90	78
B2/B3/B4						
Combinations: 1,2-dioctanoyl-sn- glycero-3-	Glycyrrhetinic Acid	12-Hydroxystearic	81	91	95	57
phosphoethanolamide 1,2-dioctanoyl-sn-	Glycyrrhetinic	Linseed oil	81	91	103	62
glycero-3- phosphoethinolamide	Acid					
1,2-dioctanoyl-sn- glycero-3-	Glycyrrhetinic Acid	Elaidic acid	81	91	93	75
phosphoethanolamide Phosphatidyl Choline	2-Hydroxystearic	C Arachidonic Acid (Na+ salt)	88	83	78	60
B2/B3/B5 Combinations:			<u></u>	- X		
Phosphatidyl Choline	e Climbazole	Linolenic Acid	88	84	208	84
Phosphatidyl Choline		Arachidonic Acid (Na+ salt)	88 ·	- 84	78	83
1,2-dioctanoyl-sn- glycero-3-	Amsacrine-HCl	Climbazole	81	79	84	58
phosphoethanolamide 1,2-dioctanoyl-sn-	Amsacrine-HCl	7H-	81	79	94	59

glycero-3- phosphoethanolamide		a]E	zimidazo[2,1- enz[de]-					
1,2-dioctanoyl-sn- glycero-3-	Glycyrrhetinic	3,4	quinolin-7-one ,-Dihydro- H)-	81	91		90	56
phosphoethanolamide		qui	nolinone(Hydroc ostyril)					
1,2-dioctanoyl-sn- glycero-3-	Glycyrrhetinic	2-	roxyquinoline(C	81	91		95	75
phosphoethanolamide 1,2-dioctanoyl-sn-			ostyril)	81	91	1	05	72
glycero-3- phosphoethanolamide		Ben	zotriazole					Έ.
1,2-dioctanoyl-sn- glycero-3-			ryl roxyethylimidaz	81	91		52	79
phosphoethanolamide 1,2-dioctanoyl-sn-	Glycyrrhetinic	oli Que	ne rcetin	81	91	:	92	73
glycero-3- phosphoethanolamide	Acid	C1 i	mbazole	0.1	0.1		0.4	5.4
1,2-dioctanoyl-sn- glycero-3- phosphoethanolamide	Glycyrrhetinic Acid	CTT	mp45016	- 81	91	1	84	54
1,2-dioctanoyl-sn- glycero-3-	Glycyrrhetinic Acid	Clo	trimazole	81	91		79	42
phosphoethanolamide 1,2-dioctanoyl-sn-	Glycyrrhetinic	Mic	onazole	81	91	:	82	43
glycero-3- phosphoethanolamide	Acid							
B2/B4/B5 Combinations:								
Phosphatidyl Choline	.2-Hydroxystearic		Amino Benzotriazo	le	88	83	105	. 77
Phosphatidyl Choline	2-Hydroxystearic acid		Lauryl hydroxyethylimida:	zoline	88	83	52	74
Phosphatidyl Choline	acid		Quercetin		88	83	92	69
Phosphatidyl Choline	acid		Oleoyl hydroxyethlimidaz		88	83	76	75
Phosphatidyl Choline	acid		7H-Benzimidazo[2, a]Benz[de]-isoqui		88	83	94	79
Phosphatidyl Choline	Climpazole		Elaidic acid		88	84	93	81
B3/B4/B5								
Combinations:								
Elaidic acid	2-Hydroxyquinolii (Carbostyril)	ne	Carbenoxolone		93	95	88	69
Elaidic acid	2-Hydroxyquinolin (Carbostyril)				93	95	134	81
Amsacrine-HCl	Amino Benzotriazole		Linseed oil		79	105	103	45
Myristic Acid	Climbazole		12-Hydroxystearic	acid	79	84	95	81
Myristic Acid	Climbazole		Linseed oil		79	84	103	81
Elaidic acid	2-Hydroxyquinolin (Carbostyril)	ne	Arachidonic Acid	(Na+ salt)	93	95	78	63

5 Synergy of Tgase inhibition with quaternary combinations of boosters

To investigate synergistic inhibition of Tgase expression by combinations of 4 different classes of boosters with retinol, selected combinations of compounds were tested. The concentrations tested were one log order of magnitude less than the concentration required for minimal inhibition of Tgase activity (i.e. IC_{20}).

The compounds were tested alone and in combination and the % inhibition of Tgase is given for each compound and the combination. The following examples give the synergistic 10 combinations possible quaternary all in combinations B1/B3/B4/B5; B1/B2/B4/B5; B1/B2/B3/B5; (B1/B2/B3/B4; Synergy was confirmed if the difference in % B2/B3/B4/B5;). inhibition of the combination (of 4 boosters) is more than 30% that of the inhibition by 3 booster combinations (i.e. % 15 inhibition of 4 booster combo is equal to or greater than % inhibition of 3 booster combo + 30%). All the quaternary combinations of boosters shown in the table given below showed synergy.

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Compound 1	Compound 2	Compound 3	Compound 4	Quarter- nary TG (%C)	Tertiary (1-3 combo; TG %C)	Differ- ence (<30%=sy nergy)
B1/B2/B3/B4 Combin	ation:			0.1		42
Castor oil Methyl	Phosphatidyl	Glycyrrhetinic	12-Hydroxy- stearic acid	21	64	12
Ester Acid (MEA) Naringenin	Choline Phosphatidyl	Acid Glycyrrhetinic	12-Hydroxy-	15	5 57	41
Natingenin	Choline	Acid	stearic acid	-3	3 40	43
Linoleoyl Monoethanolamide (LAMEA)	<pre>1,2-dioctanoyl- sn-glycero-3- phosphoethanol-</pre>	Acid	12-Hydroxy- stearic acid	•	,	
Linoleoyl Monoethanolamide (LAMEA)	amide 1,2-dioctanoyl- sn-glycero-3- phosphoethanol-	Glycyrrhetinic Acid	Isostearic acid	!	5 40	ე 35
Linoleoyl Monoethanolamide (LAMEA)	amide 1,2-dioctanoyl- sn-glycero-3- phosphoethanol-	Amsacrine-HCl	12-Hydroxy- stearic acid	- :	3 42	2 45

•							
	amide						
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl- sn-glycero-3- phosphoethanol-	Amsacrine-HCl	Elaidic acid	8	42		34
Hexanoyl sphingosine (C6	amide TCC	Glycyrrhetinic Acid	Isostearic acid	7	54		47
Ceramide) Lyrial	TCC	Vanilin	Hexadecan- edioic acid	10	48		38
Cocoyl hydroxyethylimid- azoline	1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide	Glycyrrhetinic Acid	Isostearic acid	0	37		37
Cocoyl hydroxyethylimid- azoline	Phosphatidyl Choline	Arachidonic Acid (Na+ salt)	2-Hydroxy- stearic acid	-1	37		38
Cocoyl hydroxyethylimid- azoline	1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide	Glycyrrhetinic Acid	Linseed oil	-2	45		47
B1/B2/B3/B5 Combination:							
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	Climbazole		20	64	44
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	Clotrimazole		26	64	38
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	Miconazole		9	64	55
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	Ketoconazole		5	64	59
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	Lauryl hydroxyethylimidazolin	16	15	64	49
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	Oleoyl hydroxyethlimidazoline		2	64	61
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	7H-Benzimidazo[2,1- 5 a]Benz[de]-isoquinolis 7-one		25	64	39
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	12-Hydroxystearic acid	i	18	62	44
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	Climbazole		22	62	40
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	Clotrimazole		24	62	38
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	Miconazole		13	62	50
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	Ketoconazole		12	62	50
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	Lauryl hydroxyethylimidazoli:	ne.	14	62	49
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	Oleoyl hydroxyethlimidazoline		3	62	59
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	7H-Benzimidazo[2,1- a]Benz[de]-isoquinolin 7-one		24	62	39
Naringenin	Phosphatidyl Choline	Glycyrrhetinic Acid	Miconazole		1	57	56
Naringenin	Phosphatidyl Choline	Glycyrrhetinic Acid	Ketoconazole		22	57	34
Naringenin	Phosphatidyl Choline	Glycyrrhetinic Acid	Lauryl . hydroxyethylimidazoli	ne	10	57	46
Naringenin	Phosphatidyl Choline	Glycyrrhetinic Acid	Oleoyl hydroxyethlimidazolin		2	57	54
Naringenin	Phosphatidyl Choline	Glycyrrhetinic Acid	7H-Benzimidazo[2,1- a]Benz[de]-isoquinoli		15	57	42

		~1	7-one	. 1	30 "	41
Palmitamide Monoethanolamide	Phosphatidyl Choline	Glycyrrhetinic Acid	Miconazole	-2	39.	41
Palmitamide	Phosphatidyl	Glycyrrhetinic	Oleoyl	6	39	33
Monoethanolamide Farnesol	Choline Phosphatidyl	Acid Glycyrrhetinic	hydroxyethlimidazoline Miconazole	3	43	40
r armesor	Choline	Acid		_		
Farnesol	Phosphatidyl Choline	Glycyrrhetinic Acid	Oleoyl hydroxyethlimidazoline	6	43	37
Geraniol	1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide		Miconazole	11	47	36
Geraniol .	1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide		Oleoyl hydroxyethlimidazoline	3	47	44
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide	Glycyrrhetinic Acid	Climbazole .	2	40	37
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide	Glycyrrhetinic Acid	Miconazole	5	40	35
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide	Glycyrrhetinic Acid	Ketoconazole	0	40	40
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide	Glycyrrhetinic Acid	Lauryl hydroxyethylimidazoline	-2	40	41
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide	Glycyrrhetinic Acid	Oleoyl hydroxyethlimidazoline	5	40	35
Linoleoyl Monoethanolamide (LAMEA)	<pre>1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide</pre>	Glycyrrhetinic Acid	7H-Benzimidazo[2,1- a]Benz[de]-isoquinolin- 7-one	1	40	39
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide		Climbazole	7	42	35
Linoleoyl Monoethanolamide (LAMEA)	<pre>1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide</pre>		Clotrimazole	10	42	32
Linoleoyl Monoethanolamide (LAMEA)	<pre>1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide</pre>	Amsacrine-HCl	Miconazole	5	42	37
Linoleoyl Monoethanolamide (LAMEA)	<pre>1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide</pre>		Ketoconazole	11	42	32
Linoleoyl Monoethanolamide (LAMEA)	<pre>1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide</pre>		Lauryl hydroxyethylimidazoline	-4	42	46
Linoleoyl Monoethanolamide (LAMEA)	<pre>1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide</pre>		Oleoyl hydroxyethlimidazoline	5	42	37
Linoleoyl Monoethanolamide (LAMEA)	<pre>1,2-dioctanoyl- sn-glycero-3- phosphoethanol-</pre>		7H-Benzimidazo[2,1- a]Benz[de]-isoquinolin- 7-one	8	42	35

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	amide					
Monoethanolamide	1,2-dioctanoyl- sn-glycero-3- phosphoethanol-	Amsacrine-HCl	Miconazole	13	43	30
	amide 1,2-dioctanoyl- sn-glycero-3- phosphoethanol-	Amsacrine-HCl	Oleoyl hydroxyethlimidazoline	3	43	40
Alpha-Damascone	amide 1,2-dioctanoyl-	Amsacrine-HCl	Miconazole	11	48	37
	sn-glycero-3- phosphoethanol- amide					
Alpha-Damascone	1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide	Amsacrine-HCl	Ketoconazole	13	48	34
Alpha-Damascone	1,2-dioctanoyl- sn-glycero-3- phosphoethanol- amide	Amsacrine-HCl	Lauryl hydroxyethylimidazoline	15	48	33
Alpha-Damascone	1,2-dioctanoyl- sn-glycero-3- phosphoethanol-	Amsacrine-HCl	Oleoyl hydroxyethlimid- azoline	3	48	45
Castor oil Methyl	amide Phosphatidyl	Carbenoxolone	12-Hydroxystearic acid	3	55	52
Ester Acid (MEA) Castor oil Methyl	Choline Phosphatidyl Choline	Carbenoxolone	Climbazole	6	55	49
Ester Acid (MEA) Castor oil Methyl	Phosphatidyl	Carbenoxolone	Miconazole	-2	55	57
Ester Acid (MEA) Castor oil Methyl	Choline Phosphatidyl Choline	Carbenoxolone	Ketoconazole	1	55	54
Ester Acid (MEA) Castor oil Methyl	Phosphatidyl Choline	Carbenoxolone	Lauryl hydroxyethylimi- dazoline	4	55	51
Ester Acid (MEA) Castor oil Methyl	Phosphatidyl Choline	Carbenoxolone	Oleoyl hydroxyethlimidazoline	3	55	52
Ester Acid (MEA) Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Carbenoxolone	7H-Benzimidazo[2,1- a]Benz[de]-isoquinolin- 7-one	11	55	44
Naringenin	Phosphatidyl Choline	Linoleic Acid	Climbazole	-1	45	46
Geraniol	Phosphatidyl Choline	Linoleic Acid	Climbazole	1	40	39
Acetyl sphingosine		Linoleic Acid	Climbazole	. 0	40	40
(C2 Ceramide) Acetyl sphingosine	Phosphatidyl	Linolenic Acid	Climbazole	10	40	30
(C2 Ceramide) Dimethyl	Choline TCC	Amsacrine-HCl	Elaidic acid	14	47	33
imidazolinone Dimethyl	TCC	Amsacrine-HCl	Quercetin	12	44	32
imidazolinone Dimethyl	TCC	Amsacrine-HCl	Coumarin	14	58	44
imidazolinone Hexanoyl	TCC	Glycyrrhetinic Acid	Amino Benzotriazole	8	48	40
sphingosine (C6 Ceramide) Alpha-Damascone	TCC	Myristic Acid	Climbazole	10	44	34
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	B1/B2/B4/B5 Combination:						
	Lyrial	Vanilin	Hexadecanedioic	Miconazole	12	48	36
	Lyrial	Vanilin	Hexadecanedioic acid	Oleoyl hydroxyethlimidazoline	4	48	45
	Crocetin	TCC	Elaidic acid	2- Hydroxyquinoline(Carbost yril)	11	48	37
	Hexanoyl sphingosine (C6 Ceramide)	Glycyrrhetinic Acid	12-Hydroxystearic acid	Amino Benzotriazole	14	48	33
	Dimethyl imidazolinone	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo(2,1- a]Benz[de]-isoquinolin- 7-one	2	44	42
	Melinamide	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	5	44	39
	Geranyl geraniol	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1- a]Benz[de]-isoquinolin- 7-one	9	44	35
	Cocoyl hydroxyethylimidaz oline	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	-8	44	52
	Acetyl sphingosine (C2 Ceramide)	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	10	44	34
•	Crocetin	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	10	44	34
	N,N-Diethyl Cocamide (Cocamide DEA)	Phosphatidyl Choline		7H-Benzimidazo(2,1- a]Benz[de]-isoquinolin- i7-one	4	44	40
	Cocoyl hydroxyethylimidaz oline	Phosphatidyl Choline	Elaidic acid	Climbazole	-4	30	34
	B1/B3/B4/B5 Combination:			7			
	Dimethyl imidazolinone	Amsacrine-HCl	Elaidic acid	Miconazole	7	47	40
	Dimethyl imidazolinone	Amsacrine-HCl	Elaidic acid	Ketoconazole	6	47	41
	Dimethyl imidazolinone	Amsacrine-HCl	Elaidic acid	Oleoyl hydroxyethlimidazoline	3	47 .	44
	Hexanoyl sphingosine (C6 Ceramide)	Glycyrrhetinic Acid	Isostearic acid	Clotrimazole	20	54	34
	Hexanoyl sphingosine (C6 Ceramide)	Glycyrrhetinic Acid	Isostearic acid	Miconazole	10	54	43
	Hexanoyl sphingosine (C6 Ceramide)	Glycyrrhetinic Acid	Isostearic acid	Lauryl hydroxyethylimidazoline	20	54	33
	Hexanoyl sphingosine (C6 Ceramide)	Glycyrrhetinic Acid	Isostearic acid	Oleoyl hydroxye <u>t</u> hlimidazoline	5	54	48
	Crocetin	Linoleic Acid	Elaidic acid	2-Hydroxyquinoline (Carbostyril)	0	48	48
	Crocetin	Linolenic Acid	Elaidic acid	2-Hydroxyquinoline (Carbostyril)	-2	48	50
	Castor oil Methyl Ester Acid (MEA)	Linoleic Acid	Elaidic acid	2-Hydroxyquinoline (Carbostyril)	-1	31	32
	Cocoyl hydroxyethylimid- azoline	Carbenoxolone	Elaidic acid	2-Hydroxyquinoline (Carbostyril)	-6	28	34
	;						

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	B2/B3/B4/B5						
	Combination:	Clearmanatinic	: : Teostearic acid	Ketoconazole	4	37	33
	1,2-dioctanoyl-sn-glycero-3-	Acid	ISOSCERITE ACIA	No cocondition			
	phosphoethanol- amide		•				
	1,2-dioctanoyl-sn-	Glycyrrhetinic	Isostearic acid	Oleoyl	6	37	31
	glycero-3-	Acid		hydroxyethlimidazoline			
	phosphoethanol-						
	amide	Arachidonic	2-Hydroxystearic	Miconazole	6	37	31
	Phosphatidyl Choline	Arachidonic Acid (Na+ salt)		TITCONG 2010	-		
	Phosphatidyl	Arachidonic	2-Hydroxystearic	Oleoyl	5	37	32
	Choline	Acid (Na+ salt)	acid	hydroxyethlimidazoline		4.5	
	1,2-dioctanoyl-sn-	Glycyrrhetinic	Linseed oil	Miconazole	-1	45	47
	glycero-3-	Acid					
	phosphoethanolamid					•	
	1,2-dioctanoyl-sn-	Glycyrrhetinic	Linseed oil	Oleoyl	7	45	38
	glycero-3-	Acid		hydroxyethlimidazoline			
	phosphoethanol-						
	amide	0 1	2-Hydroxystearic	7H-Benzimidazo(2,1-	8	44	36
	Phosphatidyl Choline	Carbenoxolone	acid	a Benz[de]-isoquinolin-	_	• •	
	Choline			7-one			
	Phosphatidyl	Linoleic Acid	2-Hydroxystearic	7H-Benzimidazo(2,1-	-3	44	47
	Choline		acid	a]Benz[de]-isoquinolin-			
		ma	Elaidic acid	7-one Climbazole	-3	30	33
0	Phosphatidyl Choline	Glycyrrhetinic Acid	Elaluic acid	CIIMBUZOIC			
	Phosphatidyl	Linoleic Acid	Elaidic acid	Climbazole	-2	30	32
	Choline						

Cosmetically Acceptable Vehicle

The composition according to the invention also comprises a cosmetically acceptable vehicle to act as a dilutant, dispersant or carrier for the active components in the composition, so as to facilitate their distribution when the composition is applied to the skin.

Vehicles other than or in addition to water can include liquid or solid emollients, solvents, humectants, thickeners and powders. An especially preferred non-aqueous carrier is a polydimethyl siloxane and/or a polydimethyl phenyl siloxane. Silicones of this invention may be those with viscosities ranging anywhere from about 10 to 10,000,000 centistokes at 25°C. Especially desirable are mixtures of low

and high viscosity silicones. These silicones are available from the General Electric Company under trademarks Vicasil, SE and SF and from the Dow Corning Company under the 200 and 550 Series. Amounts of silicone which can be utilised in the compositions of this invention range anywhere from 5 to 95%, preferably from 25 to 90% by weight of the composition.

Optional Skin Benefit Materials and Cosmetic Adjuncts

10 An oil or oily material may be present, together with an emulsifier to provide either a water-in-oil emulsion or an oil-in-water emulsion, depending largely on the average hydrophilic-lipophilic balance (HLB) of the emulsifier employed.

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Various types of active ingredients may be present in cosmetic compositions of the present invention. Various types of active ingredients may be present in cosmetic compositions of the present invention. Actives are defined as skin or hair benefit agents other than emollients and other than ingredients that merely improve the physical characteristics of the composition. Although not limited to this category, general examples include sunscreens, skin lightening agents, and tanning agents.

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Sunscreens include those materials commonly employed to block Illustrative - compounds ultraviolet light. are the derivatives of PABA, cinnamate and salicylate. For example, octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone (also oxybenzone) Octyl known as can be used.

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methoxycinnamate and 2-hydroxy-4-methoxy benzophenone are commercially available under the trademarks, Parsol MCX and Benzophenone-3, respectively.

5 The exact amount of sunscreen employed in the emulsions can vary depending upon the degree of protection desired from the sun's UV radiation.

optional ingredient Another preferred is selected essential fatty acids (EFAs), i.e., those fatty acids which 10 are essential for the plasma membrane formation of all cells, keratinocytes EFA deficiency makes cells in Supplementation of EFA corrects this. hyperproliferative. EFA's also enhance lipid biosynthesis of epidermis provide lipids for the barrier formation of the epidermis. 15 The essential fatty acids are preferably chosen from linoleic acid, y-linolenic acid, homo- y-linolenic acid, columbinic acid, eicosa-(n-6,9,13)-trienoic acid, arachidonic acid, ylinolenic acid, timnodonic acid, hexaenoic acid and mixtures 20 thereof.

Emollients are often incorporated into cosmetic compositions of the present invention. Levels of such emollients may range from about 0.5% to about 50%, preferably between about 5% and 30% by weight of the total composition. Emollients may be classified under such general chemical categories as esters, fatty acids and alcohols, polyols and hydrocarbons.

Esters may be mono- or di-esters. Acceptable examples of 30 fatty di-esters include dibutyl adipate, diethyl sebacate,

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diisopropyl dimerate, and dioctyl succinate. Acceptable branched chain fatty esters include 2-ethyl-hexyl myristate, isopropyl stearate and isostearyl palmitate. tribasic acid esters include triisopropyl trilinoleate and trilauryl citrate. Acceptable straight chain fatty esters include lauryl palmitate, myristyl lactate, oleyl eurcate and include Preferred esters cocooleate. (a blend of coco-caprylate and cococaprylate/caprate glycol myristyl ether acetate, caprate), propylene diisopropyl adipate and cetyl octanoate.

Suitable fatty alcohols and acids include those compounds having from 10 to 20 carbon atoms. Especially preferred are such compounds such as cetyl, myristyl, palmitic and stearyl alcohols and acids.

Among the polyols which may serve as emollients are linear and branched chain alkyl polyhydroxyl compounds. For example, propylene glycol, sorbitol and glycerin are preferred. Also useful may be polymeric polyols such as polypropylene glycol and polyethylene glycol. Butylene and propylene glycol are also especially preferred as penetration enhancers.

25 Exemplary hydrocarbons which may serve as emollients are those having hydrocarbon chains anywhere from 12 to 30 carbon atoms. Specific examples include mineral oil, petroleum jelly, squalene and isoparaffins.

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ingredients category of functional within the Another invention present of the cosmetic compositions A thickener will usually be present in amounts thickeners. anywhere from 0.1 to 20% by weight, preferably from about 0.5% to 10% by weight of the composition. Exemplary thickeners are cross-linked polyacrylate materials available under the trademark Carbopol from the B.F. Goodrich Company. Gums may be employed such as xanthan, carrageenan, gelatin, beans gum. Under certain and locust pectin karaya, circumstances the thickening function may be accomplished by 10 a material also serving as a silicone or emollient. instance, silicone gums in excess of 10 centistokes and esters such as glycerol stearate have dual functionality.

Powders may be incorporated into the cosmetic composition of 15 These powders include chalk, talc, Fullers the invention. earth, kaolin, starch, smectite clays, chemically modified organically modified aluminum silicate, magnesium montmorillonite clay, hydrated aluminum silicate, fumed silica, aluminum starch octenyl succinate and mixtures 20 thereof.

Other adjunct minor components may also be incorporated into the cosmetic compositions. These ingredients may include coloring agents, opacifiers and perfumes. Amounts of these 25 materials may range anywhere from 0.001% up to 20% by weight of the composition.

Use of the Composition

The composition according to the invention is intended primarily as a product for topical application to human skin, especially as an agent for conditioning and smoothening the skin, and preventing or reducing the appearance of wrinkled or aged skin.

In use, a small quantity of the composition, for example from 10 1 to 5ml, is applied to exposed areas of the skin, from a suitable container or applicator and, if necessary, it is then spread over and/or rubbed into the skin using the hand or fingers or a suitable device.

15 Product Form and Packaging

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The topical skin treatment composition of the invention can be formulated as a lotion, a fluid cream, a cream or a gel. The composition can be packaged in a suitable container to suit its viscosity and intended use by the consumer. For example, a lotion or fluid cream can be packaged in a bottle or a roll-ball applicator, or a capsule, or a propellant-driven aerosol device or a container fitted with a pump suitable for finger operation. When the composition is a cream, it can simply be stored in a non-deformable bottle or squeeze container, such as a tube or a lidded jar.

The invention accordingly also provides a closed container containing a cosmetically acceptable composition as herein defined.

CLAIMS

- 1. A skin care composition comprising:
 - a. from 0.001% to 10% of a retinoid;
- b. a combination of at least 2 retinoid boosters belonging to classes B1 to B5 in an amount of from 0.0001% to 50% where the ratios of the two boosters to each other in the range of is 1:1000 to 1000:1;
 - c. a cosmetically acceptable vehicle.

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- 2. The skin care composition of claim 1 where the combination of boosters comprises at least three boosters belonging to the classes B1 to B5 in an amount of from 0.0001% to 50%.
- 3. The skin care composition of claim 1 or claim 2 where the second composition has a combination of at least 4 boosters belonging to the classes B1 to B5 in an amount of from 0.0001% to 50%.
- 20 4. The skin care composition of any of the preceding claims where the second composition has a combination of all the 5 classes of boosters belonging to the classes B1 to B5.
- 5. A cosmetic method of conditioning skin, the method comprising applying topically to the skin the product of any one of claims 1 through to 5.
- 6. A cosmetic method of mimicking the effect on skin or retinoic acid, the method comprising applying to the skin the product of any one of claims 1-5.

- 7. A skin care composition comprising:
- a. a combination of at least 2 retinoid boosters belonging to classes B1 to B5 in an amount of from 0.0001% to 50% where the ratios of the two boosters to each other in the range of is 1:1000 to 1000:1;
 - b. a cosmetically acceptable vehicle.